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AND

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WITH THE ASSISTANCE OF

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The Journal of General Microbiology

Editors: B. C. J. G. KNIGHT and A. F. B. STANDFAST

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COMPARATIVE PHYSIOLOGICAL STUDIES ON THE
GROWTH OF FIELD CROPSIII. THE EFFECT OF INFECTION WITH BEET YELLOWS AND BEET
MOSAIC VIRUSES ON THE GROWTH AND YIELD OF THE SUGAR-
BEET ROOT CROP

BY D. J. WATSON AND MARION A. WATSON

Rothamsted Experimental Station, Harpenden, Herts

(With 8 Text-figures)

Infection with beet yellows virus depressed the dry-matter yield of sugar-beet plants by decreasing both leaf area and net assimilation rate (N.A.R.). It did not reduce the number of leaves.

The lower N.A.R. of infected plants may occur because photosynthesis is slowed by chlorosis of the leaves or by changes associated with it. Plants infected at the end of June had 30-50% of their leaf area yellowed from mid-August. Later infection caused less yellowing. The yellowing almost sufficed to account for the decrease in N.A.R., if yellowed parts of leaves do not photosynthesize. However, the similar diurnal fluctuations of carbohydrate in the laminae of healthy and infected leaves suggests that photosynthesis may not be much slowed by infection; if so, the decrease in N.A.R. indicates a large increase in respiration rate, especially of the root.

The effects of yellows on leaf area and N.A.R. appear to be independent; late-sown plants suffered a greater reduction of leaf area, but a smaller reduction in N.A.R., than early-sown plants; similarly treated plants showed the same reduction of leaf area in two successive years, but the decrease in N.A.R. was much greater in the second.

Most of the loss of dry matter was in the root; the dry weight of the petiole (including stem tissue) was also decreased but the dry weight of leaf lamina was little affected.

Yellows greatly increased the reducing sugars in the leaf lamina, and caused smaller increases in sucrose and starch. These effects appeared when the leaves showed only etch symptoms. The increase in starch was greatest at this time, but the effect on sugar content subsequently increased with the development of yellowing.

Yellows reduced the water content, nitrogen content and the area/weight ratio of the leaf lamina. The change in nitrogen content, but not the others, could be accounted for by the rise in carbohydrate.

Yellows had no effect on the water content of petiole and root; it increased the nitrogen content of these parts. It reduced the sucrose content of the root, but the loss of sugar yield was mainly attributable to decreased root weight.

Infection with beet mosaic virus decreased the dry weight of sugar-beet plants only when nitrogenous fertilizer was applied. As with yellows infection, the loss of dry matter resulted from reductions in both leaf area and N.A.R. Unlike yellows, mosaic depressed the dry weight of leaf lamina, as well as of petiole and root.

Mosaic had no effect on carbohydrate content, water content or area/weight ratio of the leaf lamina, or on water content of petiole and root. It increased the

nitrogen content of all parts of plants that received no nitrogenous fertilizer, but not of nitrogen-treated plants. It slightly increased the sucrose content of the root.

Infection with beet mosaic virus at the end of June caused a 20% loss of sugar yield from plants that received nitrogenous fertilizer. Yellows infection of similar plants at the same date reduced the sugar yield by 50%.

The disease of sugar beet commonly known as 'yellows', caused by infection with beet yellows virus, is the most serious of those affecting the crop in Europe. Variation in its incidence is one of the chief factors determining the differences between years in the average yield of sugar beet in Britain. Watson, Watson & Hull (1946) showed that the yield of sugar from plants infected in June or early July was reduced to a half, or less, of that of healthy plants. The loss varied linearly with time of infection, and amounted to 3-5% of the yield of healthy plants for each week of infection between the appearance of visible symptoms and harvest. Variation in the date of sowing had little effect on the absolute loss of yield caused by infection, but as late sowing decreased the yield of healthy plants, it increased the percentage loss. Late-sown crops were more susceptible to natural infection than early sown crops. Hull & Watson (1947) found that when yield was increased by fertilizer application, the loss caused by yellows infection was also increased, approximately in proportion to the increase of yield, but when the fertilizer responses were very large the losses increased more than proportionally.

The other common virus disease of sugar beet, caused by beet mosaic virus, is less important in Britain, partly because it is not so widespread. It appears to affect yield much less than yellows, but no quantitative information on the loss that it causes has been published.

The experiments described in this paper were made to find what effects infection with beet yellows or beet mosaic virus has on the growth of sugar beet, and how the changes in growth reduce yield. In accordance with the usual growth-analysis procedure, measurements of leaf area and dry weight were made at intervals throughout the growth period, and from these the net assimilation rate, i.e. the mean rate of increase of dry matter per unit leaf area, was calculated. In this way, differences between healthy and infected plants in the progress of dry-matter accumulation leading to differences in final yield could be related to variation in the size of the assimilating system, measured by leaf area, or in its efficiency measured by net assimilation rate.

The symptoms of beet yellows and beet mosaic were described by Hale, Watson & Hull (1946). The physiological effects of infection on the host plant are largely unknown. Yellows changes carbohydrate metabolism, for infected leaves have a higher content of starch (Quanjér, 1934), sucrose and, especially, reducing sugars (Watson & Watson, 1951) than healthy leaves. Van Riemsdijk (1935) found that infection with yellows virus increased the rate of respiration of the leaves. The physiological effects of infection with beet mosaic virus have not previously been investigated.

ARRANGEMENT OF EXPERIMENTS

The plant material was grown in two experiments on adjacent sites in Long Hoos field on Rothamsted farm. Exp. 1, in 1945, tested the six combinations of the following treatments:

(a) Early sowing (1) *v.* late sowing (2).

(b) Plants not infected (—) *v.* plants infected with yellows virus early (end of June) (E) *v.* plants infected late (early August) (L).

Exp. 2, in 1946, compared all combinations of the following treatments:

(a) No nitrogenous fertilizer (—) *v.* 3 cwt. sulphate of ammonia per acre (N).

(b) Plants not infected (—) *v.* plants infected with yellows virus (Y) *v.* plants infected with mosaic virus (M).

In each experiment, the six treatment combinations were arranged in two randomized blocks, so that there were twelve plots in all. The labour involved in sampling and in infecting the plants made it impracticable to use higher replication. The variations of sowing date and infection date in Exp. 1, and the nitrogen treatment in Exp. 2, were included mainly to widen the basis of applicability of the results; they also provided additional internal replication of the primary comparisons between healthy and infected plants.

The variety of sugar beet used in both experiments was Kleinwanzleben E. Each plot was approximately 100th acre, and included seven rows 22 in. apart. The plants were 10 in. apart in the rows in Exp. 1, and 9 in. apart in Exp. 2; the actual mean spacings, determined from the harvested samples, were 10.2 and 9.1 in. giving plant populations of 27.9 and 31.5 thousands per acre, respectively.

All plots of Exp. 1 received 3 cwt. sulphate of ammonia, 5 cwt. superphosphate, 1 cwt. muriate of potash and 2 cwt. agricultural salt per acre, applied in the seed-bed before the first sowing. A similar basal fertilizer dressing was given in Exp. 2, except that sulphate of ammonia was applied only to plots of treatment N, and superphosphate application was at the rate of $3\frac{1}{2}$ cwt. per acre.

Plants on the plots of treatments E and L in Exp. 1, and of treatment Y in Exp. 2, were infected with yellows virus by means of aphids (*Myzus persicae*), by the procedure described by Watson *et al.* (1946). Infections with mosaic virus on plots of treatment M in Exp. 2 were made by rubbing one or two leaves per plant with sap from infected plants, after dusting with 'celite'. The yellows and mosaic viruses used were from stock cultures in sugar-beet plants grown in the glasshouse, and were originally obtained from infected plants collected on Rothamsted farm. The same stock culture of yellows virus was used in both experiments. On the day after infection, or 2 days after in Exp. 2, the whole experimental area was fumigated with nicotine vapour to kill the aphids. Exp. 2 was dusted with nicotine dust in late July after a small natural aphid infestation had been observed.

The dates of sowing and of infection in Exp. 2 were nearly the same as those of treatments 1 and E, respectively, in Exp. 1 of the previous year, and the rate of

nitrogen application for treatment N in Exp. 2 was the same as the basal rate in Exp. 1, so that plants receiving the treatment combinations N and YN in Exp. 2 were directly comparable with those receiving 1 and 1E, respectively, in Exp. 1.

Throughout this paper, the term 'healthy' will be used to signify 'having no symptoms of virus infection'.

Sampling procedure

Samples were taken in Exp. 1 to measure growth attributes on six occasions, at intervals of 4 weeks. There were also six samplings in Exp. 2, but the intervals were varied; the first three were each 3 weeks, and the subsequent ones, made in the later stages when growth was slower, were 4 or 5 weeks. In Exp. 1, the samples taken on the first occasion, just before the first time of infection, each consisted of twenty plants, made up of a pair of plants taken from each of the five centre rows at both ends of a plot. For the subsequent samples, each plot was divided transversely into five sections. From the centre of one section selected at random, a rectangular area of $\frac{1}{1000}$ th acre (five rows \times 57 in.) was harvested on each sampling occasion. The edge rows of the plot were left as guards, and each sample area was separated from its neighbours by a strip of non-experimental plants. A similar procedure was followed in Exp. 2, except that each plot was divided into six sections, one for each sampling occasion, and the area of a sample was slightly smaller ($\frac{1}{1056}$ th acre, five rows \times 54 in.). The mean number of plants in a sample was 28 in Exp. 1, and 30 in Exp. 2. Measurements made on samples of 20 plants at sampling time 1 in Exp. 1 were corrected so as to apply to the mean number of plants per sample found at the later sampling times. The sampling unit was defined by a constant area of crop rather than a constant number of plants, because inter-plant competition in the later stages of growth tends to compensate for variable plant number, so that sampling errors on an area basis should be smaller than on a plant-number basis. A sampling harvest usually occupied 2 days, all the plots of one block being sampled on the same day.

The time sequence of operations is shown in Table 1.

In Exp. 2, from sampling 3 onwards, some plants began to produce inflorescences. These were replaced in the samples by non-bolting plants from the same row, immediately adjoining the sampled area. The total number of bolters replaced was 22, out of over 1400 plants sampled at times 3 to 6, and half of these occurred in the last sampling.

Observations made on the samples

The plants were dug with a hand-fork, taking care to recover the whole of the storage root. The roots were cut off at the level of the lowest leaf scar, washed to remove soil and dried with a cloth. The laminae of the living leaves were cut off at about the point of insertion of the lowest vein on the midrib, and the leaves were counted, excluding those less than 1 in. long. The fraction of the plant remaining after removal of the root and leaf laminae, consisting mainly of petioles but in-

TABLE 1. *Dates of operations*

	Exp. 1	Exp. 2
Sowing	1 13 Apr. 2 24 May	15 Apr.
Thinning	1 28-30 May 2 23 June	31 May-3 June
Infection	E 25 June L 6 Aug.	M 2 July Y 3 July
Fumigation	E 26 June L 7 Aug.	5 July
Sampling	1 22 June 2 20 July 3 16-17 Aug. 4 13-14 Sept. 5 11-12 Oct. 6 7-8 Nov.	1 July 22-23 July 12-13 Aug. 2-3 Sept. 30 Sept.-1 Oct. 4-5 Nov.

cluding also the stem tissue that forms the 'crown' of the beet and, in the older samples, a few dead leaves, is subsequently referred to as 'petiole'.

The following determinations were made on each sample:

(1) Number of plants.

(2) Total number of leaves; leaves produced from the apical bud and from axillary buds were counted separately.

(3) Fresh weight of leaf lamina, petiole and root.

(4) Dry matter percentage of fresh weight in leaf lamina, petiole and root.

From (3) and (4) the total dry weight of the three fractions of the plants were determined.

(5) Mean area/fresh weight ratio of the leaf lamina. From this and the total fresh weight of leaf lamina, the total leaf area of the sample was calculated.

Subsamples for determining dry-matter content were taken as described by Watson & Baptiste (1938). Usually, 200 g. samples of root and petiole were dried, but at the first two samplings the samples were smaller. The leaf lamina taken for leaf area estimation (see below) was also used to determine dry-matter content. After drying at 100° C, the samples were preserved for determination of total nitrogen content by micro-Kjeldahl.

At samplings 3 to 6 in Exp. 1, and 2, 4, 5 and 6 in Exp. 2, 40 g. subsamples of root tissue were cut into small pieces, dropped into 200 ml. boiling 95% alcohol, extracted for $\frac{1}{2}$ hr. under a reflux condenser, cooled and stored in the alcohol. On the same occasions in Exp. 2, 20 g. of leaf lamina, taken in the same way as for leaf area estimation, was similarly treated. These samples were later used for determinations of sugar and, in the leaf lamina, starch, as described by Watson & Watson (1951). Another series of samples of leaf lamina was taken on 24-25 September to investigate the effect of infection on translocation of carbohydrate from the leaves during the night, the results of which have already been published (Watson & Watson, 1951).

Estimation of leaf area

The total leaf area of a sample was estimated as follows: After the laminae were cut off, they were dropped at random into a tray to form a thick layer, and disks of known area were cut out with a circular punch pressed into the mass at a number of random positions on the tray. In this way, all parts of the leaves had an equal chance of being included in the subsample. A known weight of the disks was taken and counted, incomplete ones being included in the count if they were of greater area than half a complete disk. The number of disks multiplied by the cross-sectional area of the punch gave the total area of the weighed subsample, and the area/fresh weight ratio was then calculated, and multiplied by the total fresh weight of lamina to give the total leaf area of the sample.

In both experiments, on all sampling occasions except the first, a punch made from a piece of $1\frac{1}{4}$ in. internal diameter steel piping (area of cross-section 8.3 sq.cm.), sharpened to a cutting edge at one end, was used. Usually a 150 g. subsample containing over 300 disks was taken, but at some of the earlier samplings when the leaves were small it was possible to take only 100 g. At the first sampling, a smaller punch, 4.1 sq.cm. in cross-sectional area, was used, and the subsample weighed 50 g.

The proportion of total leaf surface yellowed by virus infection was estimated by recording the number of yellow disks in the subsample, a disk being counted as yellowed when more than half its area was yellow. Although discrimination between green and yellow disks was arbitrary, and not made by comparison with colour standards, different observers counting the same sample produced almost identical results.

The following test was made to determine whether the punch method gives unbiased estimates of total leaf area: Sixteen samples, each consisting of five sugar-beet plants, were taken, and the leaf laminae were cut off and weighed. From each sample 50 g. subsamples of disks were cut with each of three different punches, 4.1, 8.3 and 18.2 sq.cm. in cross-sectional area, respectively, and two observers then counted independently the numbers of disks in the subsamples. The resulting estimates of the mean leaf area of the sixteen samples (Table 2) did not vary

TABLE 2. *Estimates of leaf area obtained by using punches of different sizes, based on disk counts by two observers. Each figure is the mean leaf area in sq.cm. per sample for the same sixteen samples of five sugar-beet plants*

	Area of cross-section of punch (sq.cm.)			Mean
	4.1	8.3	18.2	
Observer A	167	160	162	163 (a)
B	167	162	163	164
Mean	167 (b)	161	162	163
S.E.: (a) 1.0, (b) 3.1.				

significantly with punch-size over the range tested, though the smallest punch tended to give higher estimates than the others. The two observers obtained very similar results, indicating that the subjective errors in the count were small.

The leaf area of the same sixteen samples were also determined by a more laborious method known to be free from bias (Watson, 1937). This depends on estimating the area/weight ratio for each of a number of randomly selected leaves, by making prints of the leaves on blue-print paper, and measuring their areas with a planimeter. The mean leaf area of the sixteen samples determined by the print method was 165 sq.cm. The difference between the means obtained by the print and the punch methods, 1.9 ± 4.1 sq.cm., was far from significant, showing that means of a large number of samples estimated by the two methods tend towards the same value, and that those obtained by the punch method are also unbiased.

RESULTS

(1) *Plant population, and the efficiency of experimental control of infection*

The variation between sampling times and treatments in the number of plants harvested (Table 3) was not significant in either experiment, showing that there was no loss of plants between thinning and harvest, and that virus infection did not kill any plants.

TABLE 3. *Number of plants harvested, total of two samples*

Treatment	Exp. 1. Sampling*						Treatment	Exp. 2. Sampling						
	2	3	4	5	6	Mean		1	2	3	4	5	6	Mean
1	57(a)	58	58	54	54	56(c)	—	60(d)	64	60	58	64	57	60(e)
1E	58	49	55	59	53	55	Y	55	58	58	65	57	57	58
1L	53	57	57	60	56	57	M	60	64	57	63	58	56	60
2	55	53	60	53	63	57	N	54	58	60	66	62	61	60
2E	61	51	57	60	50	56	YN	62	58	59	62	59	60	60
2L	52	55	56	56	54	55	MN	57	63	63	54	59	59	59
Mean	56(b)	54	57	57	55	56	Mean	58(e)	61	60	61	60	58	60

L.S.D.: (a) 12.1, (b) 4.9, (c) 5.5, (d) 8.4, (e) 3.4.

* Sampling 1 is omitted because the samples consisted of a constant number of plants, twenty per plot, instead of a constant area of crop.

Table 4 gives the percentage of plants harvested that conformed to the prescribed treatments, i.e. the percentage of healthy plants on nominally healthy plots, and the percentage of plants infected with the appropriate virus on the nominally infected plots; it measures the efficiency of the methods used to infect plants, and shows the extent of natural infection.

In Exp. 1, 87% of the plants sampled from the healthy plots of sowing 1 were free from symptoms of virus infection, but on the healthy plots of sowing 2 only 78% of the plants sampled were free from symptoms, and those taken at samplings

4 and 5 contained 40% of infected plants. Watson *et al.* (1946) showed that late-sown plants are more susceptible to natural infection than early-sown plants. The natural infections were all with yellows virus; only two plants infected with mosaic were found, and these did not occur in the samples.

TABLE 4. *Number of plants conforming to prescribed infection treatment as percentage of total number harvested*

Treatment	Exp. 1. Sampling*						Treatment	Exp. 2. Sampling*					
	2	3	4	5	6	Mean		2	3	4	5	6	Mean
1	93	86	100	72	85	87	—	100	88	93	88	90	92
1E	98	98	100	100	96	96	Y	93	100	98	100	100	98
1L	—†	26	53	85	91	—	M	92	100	100	98	98	98
2	100	89	60	64	75	78	N	91	83	76	94	79	84
2E	98	100	100	97	100	98	YN	93	100	100	100	97	98
2L	—†	16	75	96	96	—	MN	97	98	100	100	100	99

* Sampling 1 in both experiments was made before the infection treatments were applied, and all the harvested plants were healthy.

† Treatment L was not applied until after sampling 2. All plants harvested at sampling 2 from these plots were healthy.

Plants infected with yellows virus on 25 June 1945 (treatment E) began to show symptoms 3 weeks later.* By 20 July, at sampling 2, intense vein-etching had developed, and the infected plots were readily distinguished by their paler colour. At the third and subsequent samplings the plants were severely yellowed. The mean percentages of infected plants in samples taken from plots of treatments 1E and 2E were 96 and 98% respectively.

Symptoms developed more slowly on plants experimentally infected on 6 August (treatment L). Most of the plants recorded as infected at sampling 3 were probably earlier natural infections, because vein-etching, which is usually the first visible sign of infection, was not observed until sampling 4. It was still present at sampling 5, when some yellowing had also developed. Yellowing was never so intense as on plants infected earlier. The steady increase throughout the experimental period in the percentage of plants recorded as infected is presumed to be due to an increase in the number of infected plants showing symptoms, and not to spread of infection to previously healthy plants, for there was no comparable increase on the uninfected plots. At the last sampling 91% of plants of treatment 1L and 96% of plants of treatment 2L were recorded as infected, and it is assumed that a similar percentage were infected at samplings 2 to 5, though many of them were symptomless. Symptoms appeared slightly earlier and were more severe in late-sown plants (2L) than in early-sown (1L).

The slower development of symptoms in the late-infected plants (L) than in the early-infected (E) must be attributed to different environmental conditions after infection. Age of plants cannot have been responsible, for symptoms of the late

infection appeared at the same rate in early- and late-sown plants, and the plants of treatment 2 L were infected at about the same age, 7 weeks from sowing, as those of treatment 1 E. Lower light intensity and shorter days at the time of late infection may have been responsible for slow symptom development; unpublished work has shown that in reduced illumination yellows symptoms are less severe.

There were fewer natural infections in Exp. 2 than in Exp. 1. On the average of all samplings 92 % of the plants on the uninfected plots without nitrogen remained healthy. The addition of nitrogen apparently increased the susceptibility to natural infection, as only 84 % of the plants in samples from the plots of treatment N were free from symptoms. Most infections on the plots not experimentally infected were with yellows virus. A few mosaic-infected plants, averaging less than 3 %, were found at the last three samplings.

Symptoms of yellows in plants infected on 3 July began to appear by sampling 2, 22 July. Infected plants were distinguishable by their paler colour and a few also showed vein-etching. At this time, 93 % of the sampled plants were recorded as infected, but evidently some infected plants had not yet produced visible symptoms, for on the average of later samplings over 99 % of plants were infected. Intense yellowing had developed at sampling 3. Some plants eventually showed mosaic as well as yellows; the percentage of mosaic-infected plants was almost identical with that on the uninfected plots.

Symptoms of mosaic were apparent at sampling 2; in the samples taken from plots of treatments M and MN on this and subsequent occasions, 98 % of the plants were recorded as mosaic-infected. Natural infections with yellows virus occurred on the mosaic-infected plots, and increased slowly during July and August. In the samples taken at times 4 to 6, from plots of treatment M and MN, 12 % of the plants were infected with both yellows and mosaic viruses. The same mean percentage of yellows-infected plants was found in samples taken at the same times from uninfected plots. These results, and the similarity of the rates of mosaic infection on uninfected and yellows-infected plots, show that infection with mosaic virus did not affect susceptibility to yellows infection, and vice versa.

Table 4 shows that the methods of experimental infection used were reasonably effective. The lowest rate of infection achieved was 91 %, on the early-sown late-infected plots (treatment 1 L) of Exp. 1, and for all other infection treatments there were less than 5 % of failures. These failures, and the presence of naturally infected plants on nominally healthy plots, must have led to an underestimation of the effects of infection. Fortunately, the level of natural infection was well below average in both years, and the error introduced into the estimation of effects of infection was not large except on the late-sown plots in Exp. 1.

(2) Dry weight of the plant parts

The changes with time in mean dry weight per sample of leaf lamina, petiole and root are shown in Figs. 1 and 2. Means for the last three samplings are given in Table 5; these provide a more sensitive test of treatment effects than the data for individual samplings because they are less affected by sampling errors.

In Figs. 1 and 2, and subsequent figures, the magnitudes of treatment differences required to reach significance at the 5% level are represented by vertical lines.

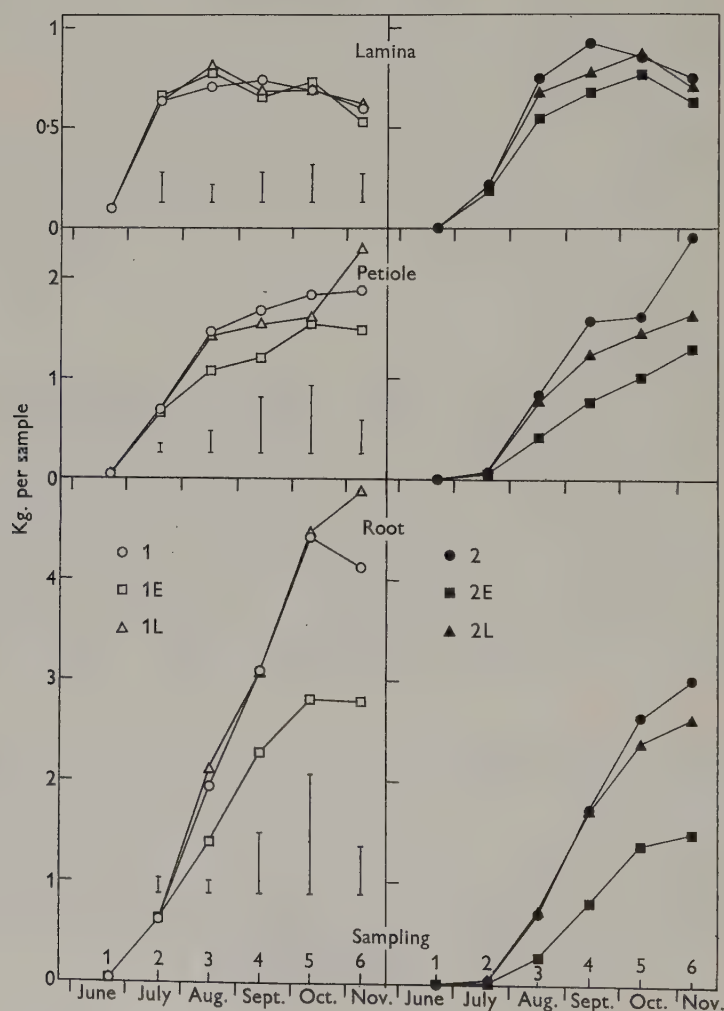


Fig. 1. Effect of infection on the dry weight of leaf lamina, petiole and root, kg. per sample; Exp. 1. (The meaning of the treatment symbols 1, 2, E and L is explained in the text.)

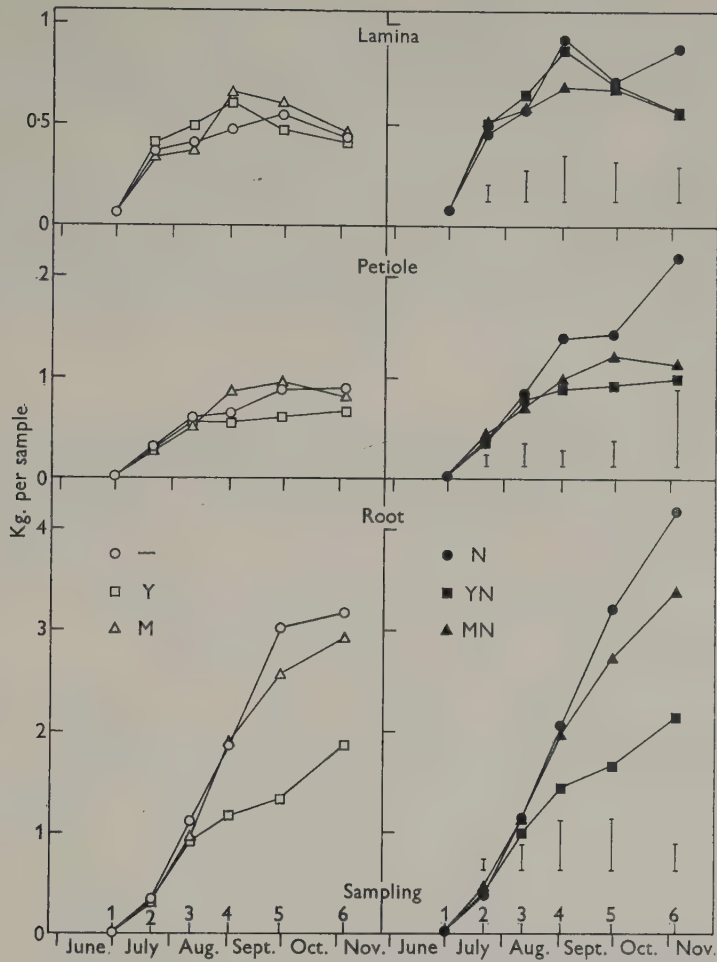


Fig. 2. [Effect of infection on the dry weight of leaf lamina, petiole and root, kg. per sample; Exp. 2. (The meaning of the treatment symbols —, Y, M and N is explained in the text.)

TABLE 5. Dry weight of plant parts, and total dry weight, kg. per sample; mean of samplings 4, 5 and 6

Exp. 1					Exp. 2				
Treatment	Lamina	Petiole	Root	Total	Treatment	Lamina	Petiole	Root	Total
1	0.67	1.79	3.87	6.34	—	0.48	0.81	2.69	3.98
1E	0.64	1.41	2.63	4.67	Y	0.50	0.61	1.45	2.56
1L	0.66	1.81	4.14	6.61	M	0.57	0.88	2.46	3.91
2	0.84	1.87	2.47	5.18	N	0.84	1.68	3.15	5.67
2E	0.69	1.04	1.24	2.96	YN	0.72	0.94	1.75	3.41
2L	0.78	1.45	2.26	4.48	MN	0.64	1.12	2.69	4.46
L.S.D.	0.05	0.20	0.28	0.46	L.S.D.	0.13	0.21	0.22	0.24

These are drawn at only one side of the figure, but apply to comparable samplings at both sides, i.e. for sowings 1 and 2 in Exp. 1 and for plots with and without nitrogenous fertilizer in Exp. 2. For variables that changed widely with time, e.g. dry weight in Figs. 1 and 2, standard errors were computed for each sampling; for others, e.g. Figs. 3 and 4, the error variances for samplings 2 to 6 were pooled. Values of the least significant difference for $P=0.05$ ($\text{L.S.D.} = \sqrt{2t} \times \text{standard error}$) are given in the tables, as well as in the figures, in preference to standard errors, because the standard errors for individual samplings are based on only 5 degrees of freedom, so that $\sqrt{2t} (P=0.05) = 3.64$ and the usual rule, that differences greater than three times the standard error may be accepted as significant, is not strictly applicable.

Experiment 1

Early yellows infection (E) had no detectable effect on lamina dry weight for sowing 1, but for sowing 2 it caused a reduction that was first apparent at sampling 3, increased at sampling 4 and subsequently decreased. It had no effect on the dry weight of petiole and root at sampling 2, when symptoms had just appeared in the leaves, but from sampling 3 onwards it caused a large reduction that increased steadily. The reduction of dry weight was relatively greater in the root than in the petiole fraction, and in late-sown plants than in early-sown.

Late yellows infection (L) had no significant effect on the dry weight of plants of sowing 1, except at sampling 6, when it apparently increased the dry weight of petiole and root. Though the increases were statistically significant, it is doubtful whether they were real treatment effects, because there was no evidence of similar increases at earlier samplings. It seems likely that, by chance, the plants sampled at time 6 from both the 1 L plots were abnormally large. Late yellows infection caused reductions in all parts of the plants of sowing 2; the reductions in lamina and petiole were significant on the average of the last three samplings (Table 4) but not for individual samplings. The effects of late infection were smaller than those of early infection and appeared later, at samplings 4 or 5 instead of 3.

Experiment 2

The effects of yellows were similar to those of the early infection in Exp. 1. The dry weight of leaf lamina was not significantly changed, except that at the last sampling the nitrogen-treated plants showed a reduction. The dry weights of petiole and root were decreased at sampling 4, and the effects increased in the later stages of the growth period. Root dry weight was relatively more reduced than petiole dry weight, and the reductions in both were enhanced by nitrogen. All these effects on dry weight appeared later in Exp. 2 than in Exp. 1. The early infection in Exp. 1 produced large effects on petiole and root dry weight at sampling 3 on 16 August, but at the corresponding sampling in Exp. 2, on 12 August, no such effects had developed, and they did not appear until 2 September.

Mosaic reduced the dry weight of all parts, including the leaf lamina, of plants that received nitrogen; it affected petiole and root less than yellows. Where no nitrogen was given, mosaic had less effect; though it reduced root dry weight (Table 5), those of lamina and petiole were slightly, but not significantly, increased.

The effects of infection on total dry weight per sample are shown for individual samplings in Table 6, and for the means of samplings 4, 5 and 6 in Table 5. These mainly reflect the variation in root dry weight. They show that the loss of dry matter caused by yellows was increased, both absolutely and relatively, by later sowing and by nitrogen, but was much reduced by delaying infection. The loss from mosaic also increased with increase in nitrogen and it was negligible in the plants without nitrogen. The losses, expressed as percentages of the dry weight of healthy plants, are shown in Table 7. They were calculated from the means for samplings 4, 5 and 6, but the final yields give very similar figures. The percentage loss of yield caused by yellows on plots receiving nitrogen in Exp. 2 was greater than on the comparable plots in Exp. 1, although effects on dry weight were apparent earlier in the season in Exp. 1.

TABLE 6. Total dry weight, kg. per sample

Exp. 1. Sampling							Exp. 2. Sampling						
Treatment	1	2	3	4	5	6	Treatment	1	2	3	4	5	6
1	0.16	1.95	4.10	5.49	6.93	6.60	—	0.11	1.03	2.13	2.99	4.44	4.51
1E	—	1.94	3.25	4.14	5.08	4.81	Y	—	1.02	1.97	2.32	2.41	2.95
1L	—	—	4.36	5.28	6.76	7.79	M	—	0.92	1.85	3.41	4.13	4.20
2	0.03	0.34	2.29	4.24	5.13	6.17	N	0.14	1.24	2.58	4.39	5.37	7.25
2E	—	0.27	1.24	2.27	3.18	3.44	YN	—	1.27	2.44	3.23	3.30	3.71
2L	—	—	2.18	3.74	4.73	4.98	MN	—	1.42	2.44	3.65	4.63	5.09
L.S.D.	—	0.32	0.36	1.10	1.90	0.70	L.S.D.	—	0.28	0.62	0.72	0.84	1.11

TABLE 7. Loss of dry matter caused by virus infection as percentage of yield of healthy plants. (Calculated on mean dry weights for samplings 4, 5 and 6)

Exp. 1				Exp. 2	
Sowing 1		Sowing 2		No N	N
Early yellows infection (E)		26	43	36	40
Late yellows infection (L)		-4	14	2	21
				Mosaic infection (M)	

As infection with yellows virus in June or early July had only small effects on lamina dry weight, but greatly reduced the dry weights of petiole and roots, the leaf laminae accounted for a larger proportion of the dry weight of infected plants than of healthy plants (Table 8). The ratio of lamina dry weight/total dry weight was not altered by late yellows infection nor by mosaic infection.

TABLE 8. *Dry weight of leaf lamina as percentage of total dry weight*

Treatment	Exp. 1. Sampling						Treatment	Exp. 2. Sampling					
	1	2	3	4	5	6		1	2	3	4	5	6
1	59	33	17	13	10	9	—	62	36	19	16	12	10
1E	—	34	24	16	14	11	Y	—	40	25	26	19	14
1L	—	—	19	13	10	8	M	—	36	20	19	15	11
2	—	65	33	22	17	12	N	60	37	22	21	13	12
2E	—	68	44	30	24	18	YN	—	40	27	27	21	15
2L	—	—	31	21	18	14	MN	—	36	24	19	15	11

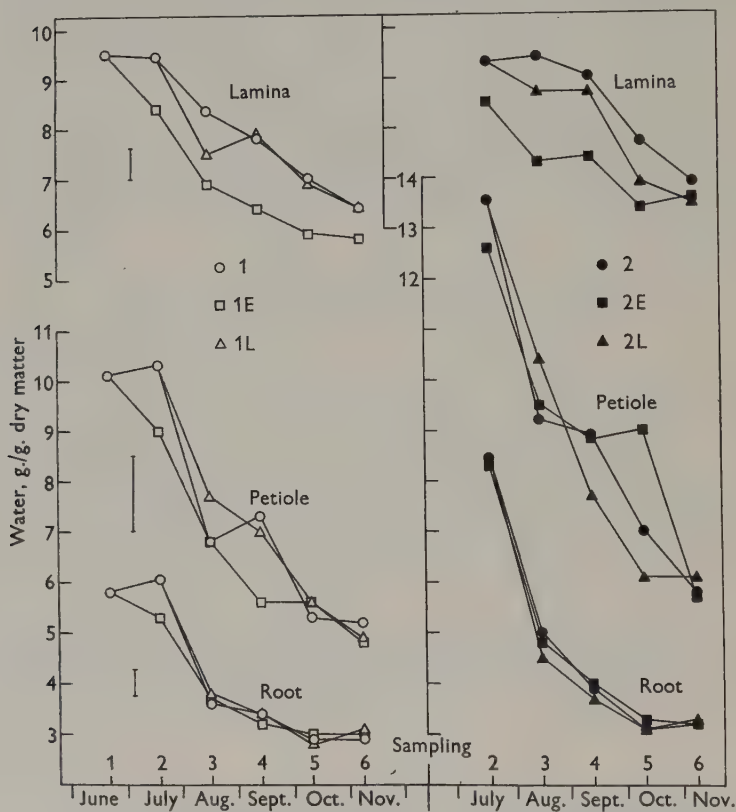


Fig. 3. Effect of infection on water content, g. per g. dry weight, of leaf lamina, petiole and root; Exp. 1.

(3) *Water content* (Figs. 3 and 4)

The water content (ratio of water/dry matter) of all parts of the plant fell sharply with age. Late-sown plants therefore had a higher water content than early-sown at all sampling times (Fig. 3), but by the end of the growth period the difference was small. Nitrogenous fertilizer slightly increased water content (Fig. 4).

The only consistent effect of virus infection was the reduction in water content of the leaf lamina by early yellows infection (E in Exp. 1, Y in Exp. 2). This change was apparent at sampling 2 before the leaves became chlorotic. It increased at samplings 3 and 4 but subsequently decreased, so that the fall with time in water

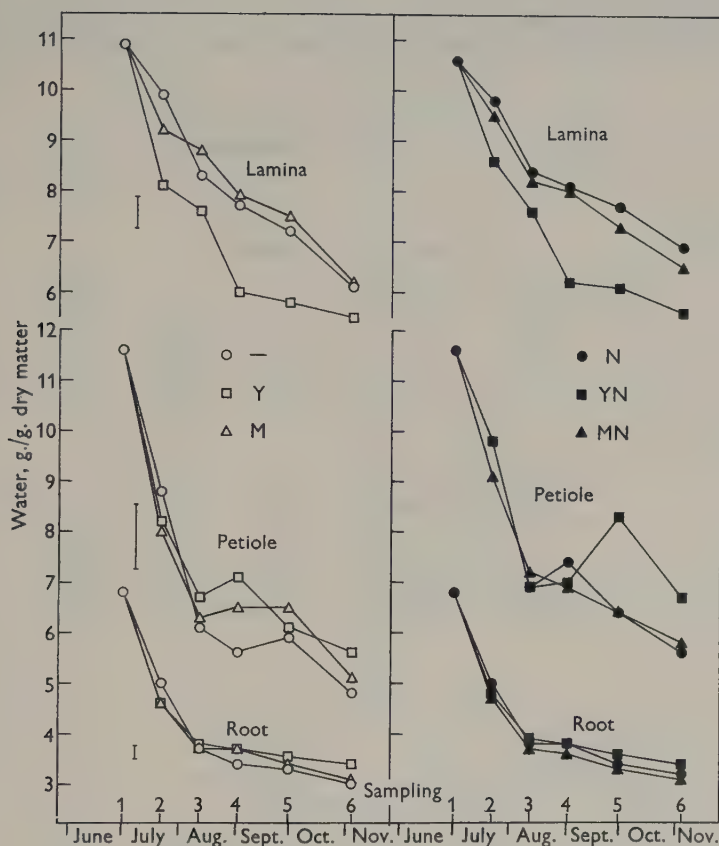


Fig. 4. Effect of infection on water content, g. per g. dry weight, of leaf lamina, petiole and root; Exp. 2.

content was at first more rapid, but later slower, in infected leaves than in healthy leaves. The decrease in water content was almost the same in early-sown and late-sown plants (Fig. 3) and was not much affected by nitrogen (Fig. 4). Late infection (L, Fig. 3) caused a similar but smaller change only in late-sown plants.

In Exp. 2, but not in Exp. 1, yellows slightly increased the water content of the root and, less regularly, of the petiole from September onwards.

Infection with mosaic virus (Fig. 4) did not significantly affect the water content in any part of the plant.

(4) *Area/weight ratio of the leaf lamina*

The area/fresh weight ratio of the leaf lamina increased between samplings 1 and 3, but subsequently changed little (Table 9*a*). It was slightly decreased by later sowing (Exp. 1).

Yellows infection fairly consistently reduced the area/fresh-weight ratio in both experiments, though only treatment 1E in Exp. 1 had a significant effect. This

TABLE 9. *Area/weight ratio of leaf lamina*

Treatment	Exp. 1. Sampling						Mean (3 to 6)
	1	2	3	4	5	6	
	(a) sq.cm. per g. fresh weight						
1	14.0	18.0	20.1	20.8	20.9	20.9	20.7
1E	—	16.2	19.0	18.7	18.4	17.4	18.4
1L	—	—	19.8	22.4	19.7	19.2	20.3
2	—	16.2	19.0	18.6	19.8	20.2	19.4
2E	—	15.0	17.9	17.9	18.2	18.8	18.2
2L	—	—	19.6	18.0	17.8	18.2	18.4
L.S.D.			1.82				1.66
	(b) sq.cm. per g. dry weight						
1	147	187	190	183	168	155	174
1E	—	153	151	138	128	118	134
1L	—	—	168	199	155	143	166
2	—	172	196	185	172	160	178
2E	—	144	148	137	134	143	140
2L	—	—	190	174	140	137	160
L.S.D.			19.2				8.0
Treatment	Exp. 2. Sampling						Mean (3 to 6)
	1	2	3	4	5	6	
	(a) sq.cm. per g. fresh weight						
—	14.4	17.6	19.0	18.2	20.0	20.4	19.4
Y	—	17.3	16.6	17.2	19.9	18.8	18.1
M	—	18.4	17.4	18.5	20.0	20.4	19.1
N	14.2	17.7	18.8	17.8	21.4	21.0	19.8
YN	—	17.9	17.6	19.4	20.6	20.0	19.4
MN	—	18.4	18.9	18.4	20.6	21.6	19.9
L.S.D.			2.38				1.71
	(b) sq.cm. per g. dry weight						
—	171	190	177	158	164	144	161
Y	—	158	143	121	134	122	130
M	—	188	171	164	170	148	163
N	165	192	177	163	187	167	174
YN	—	172	152	140	148	131	142
MN	—	193	174	166	170	162	168
L.S.D.			21.0				19.7

presumably implies that yellows-infected leaves were slightly thicker than healthy leaves, which may partly explain the fact that they feel stiffer, and easily break if they are bent—a character commonly used for diagnosis.

As yellows infection reduced the water content of the leaf lamina, it decreased the area per g. dry weight (Table 9*b*) relatively more than the area per g. fresh weight. All the effects of yellows-infection treatments on the area/dry-weight ratio were significant, but that of late infection on early-sown plants was small compared with the others.

Infection with mosaic virus did not detectably change either area per g. fresh weight or area per g. dry weight.

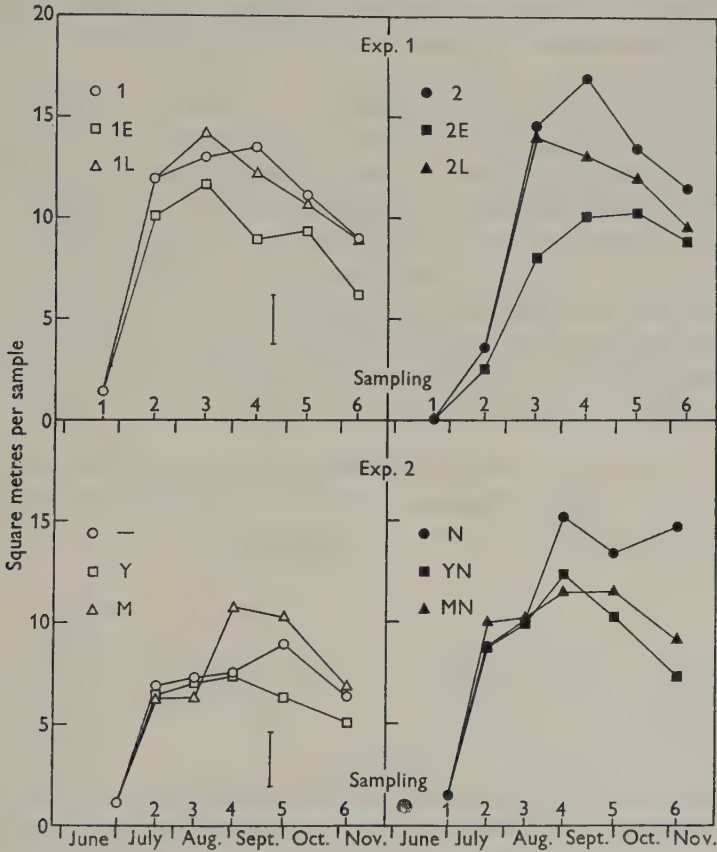


Fig. 5. Effect of infection on leaf area, sq. metres per sample.

(5) Leaf area (Fig. 5)

Late-sown plants had a larger leaf area than early-sown plants from September onwards (Exp. 1) as Watson & Baptiste (1938) showed. Nitrogen increased leaf area throughout the experimental period (Exp. 2).

Although yellows infection had only small effects on the dry weight of leaf lamina, it greatly reduced leaf area per sample. In Exp. 1 the leaf area of plants of both sowings infected at the early date (1E and 2E) was already smaller than that of healthy plants in mid-July, and the difference subsequently increased and persisted to the end of the experiment. Late yellows infection (L) reduced the leaf area only of late-sown plants. In Exp. 2 leaf area was not reduced until sampling 4, much later than in comparable plants infected at the early date in Exp. 1. At the lower level of nitrogen, the decrease in leaf area was small and not significant.

Where nitrogenous fertilizer was applied, mosaic decreased leaf area almost as much as did yellows at sampling 4, 5 and 6, but it increased the leaf area of plants that did not receive nitrogenous fertilizer, significantly at sampling 4 but not at other times. This interaction between mosaic infection and nitrogen supply requires further investigation.

In neither year was any yellowing observed at sampling 2, but it developed rapidly in the next interval (Table 10). In Exp. 1, both early- and late-sown plants infected at the early date (1E and 2E) had one-third of their leaf area yellowed at sampling 3 in mid-August. There was a slow increase later to a maximum of 40% at sampling 5, and then a slight fall. In Exp. 2 there was more extensive yellowing. At sampling 3, plants that received no nitrogenous fertilizer (Y) had half their leaf area yellowed, but the proportion subsequently fell to the same final value as in Exp. 1. Nitrogen delayed yellowing; at sampling 3 only 27% of the lamina area of plants of treatment YN was yellowed compared with 49% for treatment Y, but at all the later times the results for these two treatments were identical.

TABLE 10. *Yellowed leaf area as percentage of total leaf area*

Treatment	Exp. 1. Sampling				Treatment	Exp. 2. Sampling			
	3	4	5	6		3	4	5	6
1	4	0	12	11	—	7	5	10	10
1E	31	33	38	37	Y	49	47	45	36
1L	10	5	16	17	M	10	10	11	6
2	3	9	12	15	N	4	9	8	7
2E	33	32	41	34	YN	27	47	45	36
2L	5	4	24	28	MN	4	2	13	7
L.S.D.			8.4		L.S.D.			11.8	

The differences between seasons in the effects of yellows on dry weight and total leaf area were not closely correlated with the extent to which the leaves were yellowed; the higher proportion of yellowing in August and September in Exp. 2 was associated with smaller decreases in dry weight and leaf area at this time than in Exp. 1.

Late yellows infection (Exp. 1) never caused as much yellowing as early infection, even at the last sampling. Its effect on early-sown plants (1L) was small and not significant. The yellowing of the leaves recorded on healthy and mosaic-infected

plots, which increased slowly to about 10% in November, is attributable to natural infections with yellows virus, and perhaps partly to senescence.

The fall in the percentage of leaf area yellowed, throughout the later part of the growth period in Exp. 2, confirms the impression gained from field observations that in some conditions naturally infected crops that previously showed intense yellowing can become much greener at the end of the season.

(6) *Leaf number* (Fig. 6)

Yellows infection did not consistently change the number of living leaves present per plant. It sometimes increased but never reduced leaf number, so the reduction in total leaf area arose because the leaves were smaller. Leaf expansion was diminished by yellows infection but meristematic activity was not retarded.

The only significant effect of yellows occurred with treatment 1E in Exp. 1. This increased the number of leaves on the main axis, and treatments Y and YN in Exp. 2 appeared to have similar but smaller effects, except at the last sampling. Whether this resulted from increase in the rate of production or in the longevity of leaves is not known. The same treatments fairly consistently increased the number of leaves produced from lateral buds, but this was so variable between samples that no treatment effects were significant.

Mosaic infection increased leaf number on the main axis only in plants that received no nitrogenous fertilizer. The additional number of leaves was sufficient to account for the increased leaf area of these plants, without change in leaf size.

(7) *Net assimilation rate*

The mean rate of dry-matter increase per unit leaf area, usually called net assimilation rate (N.A.R.), was calculated for each interval between samplings as

$$\frac{(W_2 - W_1)(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)(t_2 - t_1)},$$

where W_1 is the total dry weight per sample and L_1 the total leaf area per sample at time t_1 , the beginning of the sampling interval, and W_2 and L_2 the dry weight and leaf area at time t_2 , the end of the interval. As Williams (1946) pointed out, this expression gives an unbiased estimate of mean N.A.R. in the interval $t_2 - t_1$ only if W changes linearly with L , so that dW/dL is constant. This was not true for the whole experimental period, but the relation between W and L within each sampling interval was probably sufficiently close to linearity to avoid serious bias in the estimate of mean N.A.R. Also, as the growth curves of W and L for different treatments were similar, estimates of N.A.R. were probably similarly biased, so that comparisons between treatments were not greatly affected. N.A.R. was computed for each plot, and not from the treatment means of W and L , to provide estimates of error for testing the significance of treatment effects.

Table II gives two long-period means for each treatment: A, for the whole period of the experiment; and B, for the interval between samplings 2 and 6, omitting the first period before the appearance of symptoms of virus infection in the leaves.

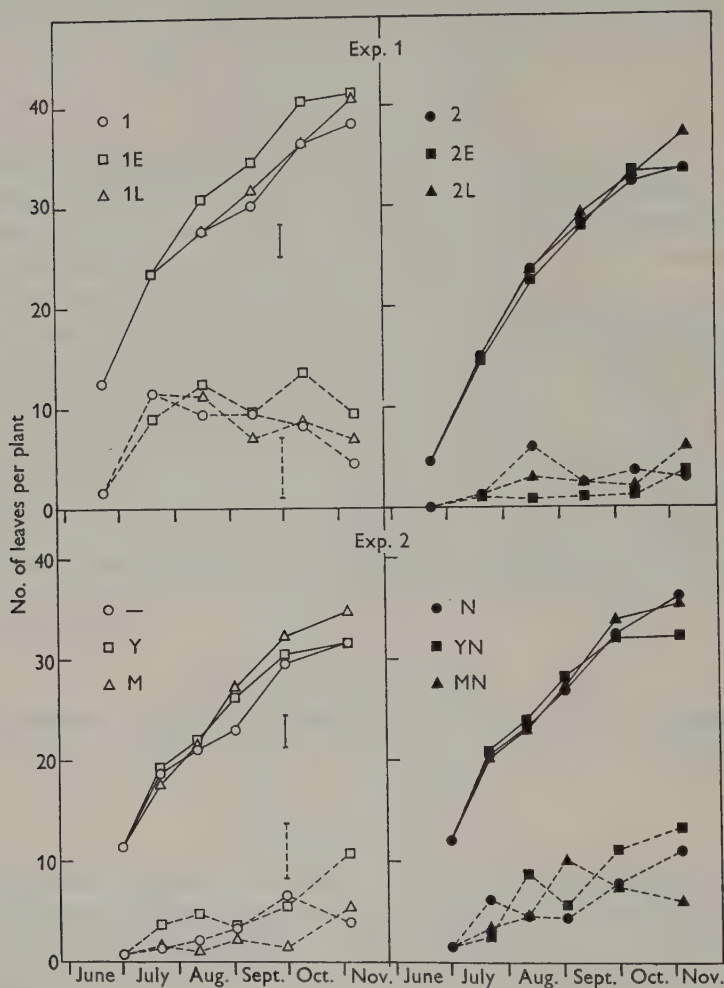


Fig. 6. Effect of infection on the mean number of living leaves per plant; full lines: leaves on the main axis produced from the apical bud; broken lines: leaves produced from lateral buds.

In both years N.A.R. fell steadily from values near 100 g. per sq.m. per week in July to about 10 in October. In Exp. 1, the late-sown plants had a higher N.A.R. than the early sown. Averaging the results for healthy and infected plants, 6 weeks delay in sowing increased mean N.A.R. for the period July–November from 38 to

44 g. per sq.m. per week; this agrees with earlier results (Watson, 1947), which showed that the mean increase of N.A.R. by delaying sowing for 1 week was 1.2 g. per sq.m. per week. This effect of varying sowing date indicates that N.A.R. decreases with advancing age of the plant. Nitrogenous fertilizer had no detectable effect on N.A.R. in Exp. 2.

TABLE 11. *Net assimilation rate; g. per sq.m. per week*

Treatment	Sampling interval					Mean*	
	1-2	2-3	3-4	4-5	5-6	A	B
	Exp. 1						
1	94(a)	44	26	28	-8	37(b)	23(c)
1E	102	30	22	26	-9	34	17
1L	87	46	17	34	27	42	31
2	99	62	31	15	21	46	32
2E	102	51	28	22	8	42	27
2L	97	61	29	20	7	43	29
Mean	97(d)	49	26	24	8	41	27
Mean of sowings 1 and 2							
—	97(e)	53	29	22	7	41(f)	27(g)
E	102	40	25	24	0	38	22
L	92	54	23	27	17	42	30
L.S.D.†: (a) 12.0, (b) 5.3, (c) 4.7, (d) 14.4, (e) 8.5, (f) 3.8, (g) 3.3.							
	Exp. 2						
	1-2	2-3	3-4	4-5	5-6	A	B
—	91(a)	52	39	44	3	41(b)	31(c)
Y	104	47	16	4	19	34	20
M	97	50	63	17	2	39	28
N	98	47	49	17	27	44	33
YN	82	42	24	1	10	28	17
MN	103	34	38	21	9	36	23
Mean	96(d)	45	38	17	12	37	25
Mean of N treatments							
—	95(e)	50	44	31	15	42(f)	32(g)
Y	93	44	20	2	14	31	18
M	100	42	51	19	5	38	25
L.S.D.†: (a) 15.3, (b) 6.7, (c) 9.6, (d) 9.8, (e) 10.8, (f) 4.8, (g) 6.8.							

* A, mean of all intervals; B, mean excluding interval 1-2. In computing the means in Exp. 2, the values for different intervals were weighted according to the length of the interval.

† All estimates of L.S.D., except (d), are appropriate only for comparisons between treatments within intervals; (d) is appropriate for the comparison of means of all treatments for the different intervals.

In Exp. 1, early yellows infection had no effect on N.A.R. in the first sampling interval, but after the leaves began to show symptoms at sampling 2, it consistently caused a reduction, except for sowing 2 in interval 4-5. The greatest reduction occurred in interval 2-3, immediately after the appearance of leaf symptoms. The means for the period between samplings 2 and 6 (B, Table 11) but not those for the

whole experimental period (A, Table 11) show a significant difference in N.A.R. between healthy and early-infected plants. Late yellows infection apparently depressed N.A.R. in interval 3-4, when leaf symptoms were beginning to appear, but afterwards it tended to increase N.A.R.

Yellows infection in Exp. 2, as in Exp. 1, had no effect on N.A.R. in the interval before the appearance of leaf symptoms at sampling 2. Subsequently it always caused a depression which reached its maximum in September (interval 4-5), somewhat later than in Exp. 1, and almost disappeared in the last interval. The mean reduction in N.A.R. over the whole experimental period was about three times that caused by the corresponding early infection in Exp. 1.

Mosaic significantly reduced mean N.A.R. for interval 2-6, but not in interval 1-2. The effect was about half as great as that of yellows infection and varied less with time. The effects of both yellows and mosaic on N.A.R. were apparently increased by nitrogenous fertilizer, but the interaction between infection and nitrogen was not significant.

(8) *The relative magnitudes of the effect of infection on leaf area and net assimilation rate*

Infection with either yellows or mosaic viruses reduced both the size of the photosynthetic system, measured by leaf area, and its activity, measured by N.A.R. The relative contributions of the two effects to the loss of dry matter can be compared if they are expressed on a percentage basis. This is done in Table 12, which refers to the period after sampling 2 when symptoms of infection and effects on growth began to appear. For the late infection in Exp. 1, which was not made until after sampling 2, the intervals between samplings 3 and 6 would be more appropriate, but the uniform use of the longer period makes no appreciable difference to the conclusions.

In calculating the mean leaf area in the period between samplings 2 and 6 (column 4, Table 12) it was assumed that leaf area changed linearly with time in each sampling interval, and the values given are therefore slightly less than the true means. However, a more accurate method of integrating the leaf area growth curves used on the data of Exp. 1, but inapplicable to Exp. 2 because of the unequal sampling intervals, gave estimates of percentage reduction of mean leaf area by infection that differed little from those tabulated.

In Exp. 1, yellows infection caused a percentage reduction of leaf area as great as, or greater than, that in N.A.R., but in Exp. 2 the reduction of leaf area was less than half that of N.A.R. Comparison of treatment 1E and 2E (Exp. 1) shows that yellows reduced leaf growth in late-sown plants more than in early-sown, but the early-sown plants suffered a greater decrease in N.A.R. Delay in infection of late-sown plants (comparison of 2L with 2E) did not greatly change the relation between the effects on leaf area and N.A.R. The apparent increase in dry-matter production caused by treatment 1L was wholly attributable to an increase in N.A.R.,

but it is doubtful whether these increases were real, though statistically significant. Nitrogenous fertilizer in Exp. 2 increased the percentage effects of yellows infection on both leaf area and N.A.R., without appreciably altering the relation between them.

TABLE 12. *The relative contributions of change in leaf area and in N.A.R. to the effect of infection on dry-matter accumulation during the interval between samplings 2 and 6*

Treatment	Increment of total dry weight		Leaf area		Net assimilation rate	
	Mean, kg. per sample	Percentage reduction by infection	Mean, sq.m. per sample	Percentage reduction by infection	Mean, g. per sq.m. per week	Percentage reduction by infection
Exp. 1						
I	4.7	—	12.0	—	23	—
1 E	2.9	38	9.5	21	17	24
1 L	5.8	-23	11.8	2	31	-35
2	5.8	—	13.1	—	32	—
2 E	3.2	45	8.5	35	27	16
2 L	4.7	19	11.4	13	29	9
L.S.D.	0.62		1.56		4.7	
Exp. 2						
—	3.5	—	7.6	—	31	—
Y	1.9	46	6.5	14	20	35
M	3.3	6	8.6	-13	28	10
N	6.0	—	12.9	—	33	—
YN	2.4	60	10.0	22	17	48
MN	3.7	38	10.7	17	23	30
L.S.D.	1.32		1.41		9.6	

The small decrease in dry-weight increment caused by mosaic infection at the lower level of nitrogen supply (treatment M, Exp. 2) resulted from a reduced N.A.R., partly offset by an increase in mean leaf area, but none of these effects was significant. Where nitrogenous fertilizer was applied, mosaic infection, like yellows infection, caused a percentage reduction in N.A.R. twice as great as that in leaf area.

The relation between the effects of yellows infection on leaf area and on N.A.R. was obviously far from constant, and its wide variation within and between experiments shows that restriction of leaf growth was not a direct consequence of reduced assimilation. If N.A.R. fell to zero, leaf growth would eventually cease because there would be no material to form new tissue, but yellows infection evidently does not act in this way. There is no deficiency of structural material in yellows-infected leaves; they have a much higher content of readily available carbohydrate than healthy leaves (Table 14).

Treatments 1 and 1 E in Exp. 1 were nearly identical with treatments N and YN respectively, in Exp. 2, and consequently differences between the effects of yellows

calculated from these two pairs of treatments can be attributed to seasonal variation in weather. Table 12 shows that the percentage reduction of mean leaf area was the same, but that of N.A.R. was twice as great, in 1946 as in 1945. With only two years to compare, it is not safe to conclude that the effect of yellows on leaf area varies less from season to season than the effect on N.A.R., but evidently the two effects are independent in their relation to climatic factors. The mean N.A.R. of infected plants (treatments 1E and YN) was the same in both years, so the greater percentage reduction in 1946 was due to the higher N.A.R. of healthy plants (treatment 1 and N).

TABLE 13. *Summary of the Rothamsted meteorological records for 1945 and 1946*

Temperature (° F.)		July	Aug.	Sept.	Oct.
Mean daily maximum	1945	69.5	67.5	63.3	60.1
	1946	70.7	66.5	63.4	54.7
Mean daily minimum	1945	53.4	52.8	52.0	44.9
	1946	52.0	50.4	50.3	44.3
Mean daily range	1945	16.1	14.7	11.3	15.2
	1946	18.7	16.1	13.1	10.4
Total rainfall (inches)	1945	2.68	1.27	2.10	2.86
	1946	2.03	3.74	3.75	1.47
Mean number of hours of bright sunshine per day	1945	5.84	4.74	2.22	3.41
	1946	7.05	4.87	3.66	2.75
Number of dull days (with less than 1 hr. bright sunshine)	1945	3	10	16	10
	1946	1	5	8	15

The meteorological records (Table 13) show that although more rain fell in the period August–October in 1946 than in 1945, there was more sunshine, and fewer dull days, especially in August and September. This may imply that N.A.R. of infected plants is less responsive to increased illumination than that of healthy plants. Watson (1947) showed that N.A.R. of sugar beet increases with decrease in the daily temperature range. Although the mean daily range in October was lower in 1946 than in 1945, in August and September it was higher, and it therefore seems unlikely that temperature differences between the two years could account for the higher N.A.R. of healthy plants in 1946. The results suggest that there may be important interactions between yellows infection and climatic factors, but to establish which factors are operative and the nature of their effects, plants would need to be grown in controlled conditions with independent variation of the different factors.

(9) *Carbohydrate content*

(a) *Leaf lamina* (Table 14)

Carbohydrate analyses of leaf lamina tissue were made only in Exp. 2. The samples from replicate plots at each sampling were bulked before analysis, so it is not possible to compute estimates of error.

The increase in starch content caused by yellows infection was already apparent at sampling 2, at the stage when vein-etching was beginning to show on leaves of infected plants. Indeed, it was maximal at this time, and subsequently fell when yellowing symptoms developed, to zero at the last sampling. Nitrogenous fertilizer decreased the starch content at all times, and the reduction was greater in infected than in healthy leaves at samplings 2 and 4, but less at later times.

TABLE 14. *Carbohydrate content of leaf lamina, percentage of dry matter; Exp. 2*

Sampling ...	Starch				Sucrose				Reducing sugars				Total			
	2	4	5	6	2	4	5	6	2	4	5	6	2	4	5	6
Treatment:																
—	0.6	1.0	0.6	0.4	2.7	3.4	2.4	3.5	3.8	5.3	4.9	8.3	7.1	9.7	7.8	12.3
Y	2.8	2.2	1.2	0.4	5.0	6.0	3.9	4.5	10.8	18.0	14.0	17.7	18.6	26.2	19.0	22.7
M	0.9	0.6	0.5	0.3	2.5	3.1	2.9	3.3	3.9	6.3	7.6	7.6	7.2	9.9	11.0	11.3
N	0.4	0.5	0.3	0.4	2.6	2.9	2.6	3.8	4.4	8.3	5.5	11.3	7.4	11.7	8.4	15.4
YN	1.5	1.3	1.1	0.4	3.3	4.4	5.3	4.3	8.4	15.3	14.4	19.2	13.2	21.0	20.8	23.9
MN	0.5	0.6	0.6	0.3	2.7	2.5	3.4	4.4	4.5	6.6	9.0	9.5	7.7	9.6	12.9	14.3

Unlike the starch content, the sugar content of the leaf lamina increased with age. The increase was mainly in reducing sugars, which more than doubled between samplings 2 and 6, but sucrose also rose appreciably. Nitrogenous fertilizer reduced sugar content at sampling 2, but later increased it. Yellows infection increased both sucrose and reducing sugars at all times, including sampling 2 when the leaves showed only etch symptoms, but later when yellowing appeared the increase became larger. Mosaic infection had no consistent effect on starch or sugar content.

The total carbohydrate content (sum of starch and sugars) of the leaf lamina was approximately doubled by yellows infection. By far the greatest increase was in reducing sugars. At the last sampling they amounted to more than one-sixth of the dry matter in yellows-infected leaves. Even higher proportions were recorded (Watson & Watson, 1951) in material taken from the same experiment in late September, just before sampling 5, when reducing sugars accounted for nearly a quarter of the dry matter in leaves of nitrogen-treated plants. The higher figure is probably due to the fact that the leaf samples were taken from selected older leaves, whereas the data in Table 14 are mean values for all leaves present on the plant, and young leaves contain less reducing sugar than old leaves.

(b) Root

Early yellows infection decreased the sucrose content of the root percentage of dry matter (Table 15); the decrease in Exp. 1 was much greater in late-sown than in early-sown plants, and in Exp. 2 it was similar for both levels of nitrogen supply. These effects on sugar content were relatively small compared with the loss of dry matter in the root; at the final sampling the largest of them, in late-sown early-

infected plants, amounted to 5% of the sucrose content of healthy plants. Mosaic infection increased the sucrose content of the root at sampling 2 (Exp. 2), but not afterwards.

The reducing sugar content of the roots varied irregularly with time, and the effects of infection were not consistent. The only significant effects of yellows shown by the means of the four samplings (Table 15) were increases by treatments 2L in Exp. 1 and Y in Exp. 2. Mosaic infection made no difference.

TABLE 15. *Sugar content of root, percentage of dry matter*

	Sucrose						Reducing sugars Mean
Sampling ...	2	3	4	5	6	Mean	
Exp. 1							
Treatment:							
I	—	71	72	74	71	72.0	0.63
1E	—	69	72	71	71	70.7	0.58
1L	—	72	72	71	74	72.3	0.52
2	—	65	71	73	73	70.6	0.53
2E	—	59	66	69	69	65.8	0.52
2L	—	64	68	72	73	69.1	0.79
L.S.D.			3.2			1.70	0.18
Exp. 2							
—	62	—	74	74	76	71.6	0.29
Y	63	—	70	73	74	70.0	0.38
M	64	—	73	74	77	72.1	0.31
N	61	—	70	74	75	70.0	0.37
YN	59	—	70	73	74	69.0	0.40
MN	65	—	71	74	76	71.3	0.37
L.S.D.			2.6			0.66	0.07

To show the effects at harvest in terms familiar to farmers and used by the factories, the sucrose content percentage of fresh weight of the root at the last sampling in each experiment is given in Table 16, together with the fresh weight of roots and the yield of sugar per acre, which is economically the most important attribute of the sugar-beet crop. Sugar content percentage of fresh weight shows the same treatment effects as on the dry-weight basis, because infection caused only small changes in water content.

Infection with yellows at the end of June depressed the sugar content of the roots by 0.4–2.3% of fresh weight, and caused a loss of 30–50% of the sugar yield. Watson *et al.* (1946) found rather larger sugar losses.

Table 16 shows that the decrease in root weight was a far more important cause of loss of sugar yield than the reduction in sugar content.

Mosaic infection decreased the yield of sugar by 10–20%. On the rare occasions when commercial beet crops have rates of mosaic infection approaching 100%,

TABLE 16. *Sucrose content of roots, percentage of fresh weight, and yield of roots and of sucrose at final harvest, in November*

Treatment	Sucrose content		Yield of roots		Yield of sucrose	
	% fresh weight	% reduction by infection	Tons per acre	% reduction by infection	Cwt. per acre	% reduction by infection
Exp. 1						
I	18.1	—	16.0	—	57.8	—
1E	17.7	2	11.0	31	38.9	33
1L	17.9	1	19.4	—21	69.4	—20
2	17.5	—	12.4	—	43.5	—
2E	16.4	7	6.3	49	20.6	53
2L	17.1	3	11.1	11	37.7	13
L.S.D.	1.3		2.1		6.4	
Exp. 2						
—	19.0	—	13.2	—	50.4	—
Y	16.7	12	8.5	36	28.5	43
M	18.9	1	12.4	6	46.9	7
N	18.0	—	18.2	—	65.6	—
YN	17.0	6	9.7	46	33.0	50
MN	18.6	—3	14.3	21	53.2	19
L.S.D.	0.5		1.2		4.1	

attained in Exp. 2, by inoculating each plant, the loss of sugar may be of economic importance, but the loss in the whole British crop is probably negligible compared with that caused by yellows.

(10) *Nitrogen content* (Figs. 7 and 8)

Replicate samples were bulked for the nitrogen estimations, so no estimates of error of treatment effects can be calculated.

Infection with yellows virus at the end of June reduced the total nitrogen content percentage of dry matter of the leaf lamina; in the petiole it produced an increase of about the same magnitude, and in the root a smaller increase. The decrease in nitrogen content of the leaf lamina occurred in both experiments at sampling 2, when leaves showed only etch symptoms and no yellowing, but the increases in petiole and root developed later, at sampling 3. Late yellows infection in Exp. 1 also depressed the nitrogen content of the leaf lamina, but made little difference to those of petiole and root. The effects of yellows infection in Exp. 2 were similar at both levels of nitrogen supply.

Mosaic infection increased the nitrogen content of all parts of plants that received no nitrogenous fertilizer, but had little or no effect in nitrogen-treated plants.

The total nitrogen uptake in g. per sample was lower in yellows-infected plants than in healthy plants (Table 17), except in early-sown plants of Exp. 1 infected at

the late date (treatment 1L) and in the low-N plants of Exp. 2, where yellows infection had no appreciable effect. Mosaic infection apparently reduced nitrogen uptake in plants that received nitrogenous fertilizer, but increased it where no fertilizer was given. On the average of samplings 4, 5 and 6 in Exp. 2 the difference in total nitrogen uptake between plants with and without nitrogenous fertilizer was

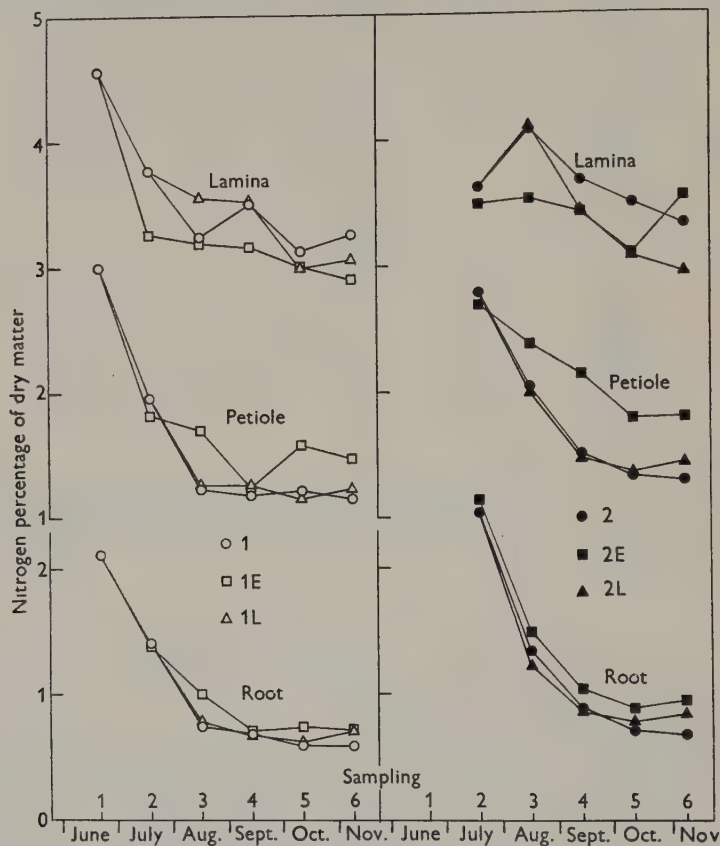


Fig. 7. Effect of infection on total nitrogen content percentage of dry matter in leaf lamina, petiole and root; Exp. 1.

34 g. per sample in healthy plants, 17 g. in yellows-infected plants and 11 g. in mosaic-infected. As the 3 cwt. sulphate of ammonia per acre supplied 30 g. N per sample area, the estimated recovery of added nitrogen in healthy plants is evidently too high, especially at sampling 6, but it is probably safe to conclude that nitrogen recovery was greatly reduced by yellows infection, and still more by mosaic infection.

The distribution of nitrogen between leaf lamina, petiole and root was not con-

sistently altered by virus infection (Table 18). Late yellows infection appeared to reduce the percentage of total nitrogen present in the lamina and increased it in the root.

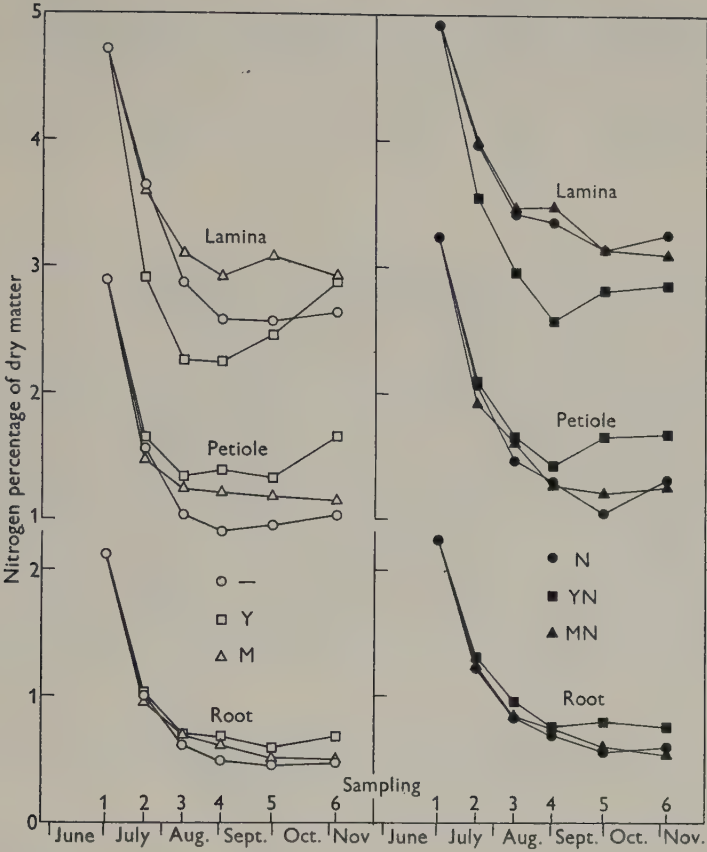


Fig. 8. Effect of infection on total nitrogen content percentage of dry matter in leaf lamina, petiole and root; Exp. 2.

TABLE 17. Total nitrogen uptake, g. per sample

Sampling ... Treatment:	Exp. 1					Treatment:	Exp. 2				
	2	3	4	5	6		2	3	4	5	6
1	46	55	67	70	65	—	22	25	27	36	36
1E	42	57	52	67	57	Y	20	25	29	28	36
1L	47	63	64	67	82	M	19	24	41	43	38
2	11	57	73	70	77	N	31	42	64	56	83
2E	9	33	48	54	60	YN	31	42	47	49	49
2L	11	52	60	65	66	MN	35	41	51	52	50

TABLE 18. *Total nitrogen in leaf lamina, petiole and root as percentage of nitrogen in whole plant; means for samplings 4, 5 and 6*

Treatment	Exp. 1			Treatment	Exp. 2		
	Lamina	Petiole	Root		Lamina	Petiole	Root
I	33	32	35	—	38	24	38
1 E	33	35	32	Y	40	29	31
1 L	30	31	39	M	42	26	32
2	40	35	25	N	41	31	28
2 E	42	36	22	YN	41	31	28
2 L	39	32	29	MN	41	27	32

(II) *Relation between the changes in carbohydrate content and in other attributes of yellows-infected leaves*

The rise in carbohydrate content in yellows-infected leaves must inevitably reduce the percentage content of other constituents, and so might account for the depressions of water and nitrogen content percentage of dry matter. Similarly, the presence of additional carbohydrate could explain the fall in the area/dry-weight ratio. If carbohydrate increase were the only absolute change in composition of the leaf lamina induced by yellows, the relative proportions of other constituents should be unaltered. To test this possibility, water and nitrogen contents and the area/dry-weight ratio in Exp. 2, recalculated on the basis of residual dry matter (R.D.M. = total dry matter less starch and sugars), are given in Table 19.

The fall in nitrogen content of the leaf lamina is accounted for almost completely by the rise in carbohydrate, but not the decreases in water content or in the area/dry-

TABLE 19. *Data from Exp. 2, expressed on the basis of residual dry matter (R.D.M.), instead of total dry matter*

	Sampling									
	2	4	5	6	Mean	2	4	5	6	Mean
	Lamina									
	Water, g. per g. R.D.M.					Area sq.cm. per g. R.D.M.				
—	10.6	8.5	7.8	7.0	8.5	205	175	178	164	180
Y	10.0	8.2	7.1	7.1	8.1	194	164	165	158	170
N	10.6	9.2	8.4	8.2	9.1	207	185	204	197	198
YN	9.9	7.9	7.8	7.3	8.2	198	177	187	172	184
	Nitrogen, percentage of residual dry matter									
	Lamina					Root				
—	3.9	2.9	2.8	3.0	3.1	2.7	1.9	1.7	2.0	2.1
Y	3.6	3.0	3.0	3.7	3.3	2.9	2.3	2.2	2.6	2.5
N	4.3	3.8	3.4	3.8	3.8	3.2	2.3	2.2	2.5	2.5
YN	4.1	3.3	3.6	3.8	3.7	3.3	2.6	3.0	3.0	2.9

weight ratio. On the mean of all samplings, nitrogen percentage of R.D.M. was nearly the same for healthy and infected plants, though infection caused small decreases at samplings 2 and 4 and increases at samplings 5 and 6. Water content and leaf area per g. R.D.M., however, were consistently reduced by yellows.

Table 19 also shows that the increase by yellows infection in nitrogen percentage of dry matter in the root in Exp. 2 was greater than could be accounted for by the reduction in sugar content, because the increase persisted when nitrogen content was referred to R.D.M. instead of total dry matter. The same result was found in Exp. 1.

DISCUSSION

The results of the two experiments show that the loss of dry matter in sugar-beet plants by infection with yellows virus arose from decreases in both leaf area and in net assimilation rate. The relative importance of the two effects varied with the age of the plant at infection and with external conditions. The yield of sugar from the roots was decreased mainly because root weight was reduced, but there was also a small decrease in the percentage sugar content.

Leaf area was reduced because the leaves were smaller, not fewer. Whether leaves were smaller because of change in cell number or cell size is unknown. It was not because of lack of assimilate, for infected leaves have more starch and sugars than healthy leaves. If yellows virus, like all the plant viruses that have been purified, is a nucleoprotein, it may diminish leaf growth by disturbing nitrogen metabolism. If virus multiplication diverted so much nitrogen from normal metabolic paths that an appreciably smaller fraction were available for growth processes, the nitrogen content percentage of dry matter might be expected to rise. However, yellows infection did not increase the nitrogen content of the leaf lamina, even when allowance was made for the additional carbohydrate by referring nitrogen content to residual dry matter. If reduced leaf growth in infected plants originates from deranged nitrogen metabolism, the change must be more complicated than merely a reduction of the internal supply of nitrogen for growth processes by diversion of nitrogen to form virus protein or other abnormal nitrogen compounds.

The net assimilation rate represents the excess of the rate of gain of dry matter by photosynthesis in the leaves over the rate of loss by respiration of the whole plant, per unit leaf area. The fall in N.A.R. caused by yellows infection could, therefore, be brought about by decrease in the rate of photosynthesis, or by increase in the rate of respiration, or by a decrease in the size of the photosynthetic system relative to the amount of respiring material, i.e. by a decrease in the ratio of leaf area to plant dry weight. The third possibility can be ruled out, for the data given in Fig. 5 and Table 6 show that yellows infection slightly increased the leaf area/total dry-weight ratio.

The yellowing of the leaves suggests that photosynthesis may be reduced by destruction of chlorophyll, or by associated changes in the chloroplasts. The greatest decrease in N.A.R. that could be brought about in this way would occur if

photosynthesis were completely inhibited in the yellowed parts of the leaves. Whether the extent of yellowing was sufficient to account for the observed reduction in N.A.R. can be estimated by recalculating N.A.R. on the basis of green leaf area instead of total leaf area; if it is assumed that yellowed parts of infected leaves did not photosynthesize, and that the rate of photosynthesis of green parts was the same as that of healthy leaves, N.A.R. on a green leaf area basis should be unaffected by infection. Table 20 shows that the means of N.A.R. in g. per week per sq.m. of green leaf area for healthy plants and for plants infected at the early date in Exp. 1 were not significantly different. Similarly, in Exp. 2, mean N.A.R. on a green leaf area basis was identical for healthy and yellows-infected plants where no nitrogenous fertilizer was given, but at the higher nitrogen level it was significantly reduced by infection. These results show that if the yellowed parts cannot photosynthesize, the extent of yellowing was of the right order to account for the observed decrease in N.A.R., except in the nitrogen-treated plants of Exp. 2. In these plants the rate of photosynthesis must have decreased in the green parts as well as in the yellowed parts of infected leaves, if the reduction of N.A.R. is to be attributed solely to decreased photosynthesis.

TABLE 20. *Net assimilation rate, g. per sq.m. of green leaf area per week. Means for period between samplings 2 and 6*

Sowing ...	Exp. 1				Exp. 2		
	1	2	Mean		—	N	Mean
—	24	34	29	—	33	35	34
E	24	38	31	Y	33	24	28
L.S.D.	7.2		5.1		9.5		6.7

However, there are reasons for doubting that yellows infection greatly reduces the rate of photosynthesis. Watson & Watson (1951) found that the loss of carbohydrate percentage of residual dry matter from the leaves between sunset and sunrise was nearly the same in yellows-infected as in healthy plants. If this is true generally, and not merely for the particular days on which the experiments were done, the gain in carbohydrate content during the day must also be unaffected by yellows infection, though this has not been tested directly by carbohydrate analyses made on samples taken at sunrise and at sunset on the same day. It is difficult to see how yellows-infected leaves could gain as much carbohydrate during the day as healthy leaves, if they have a much lower rate of photosynthesis. It would be possible, if translocation during the day as well as photosynthesis were more rapid in healthy leaves, but it seems unlikely that yellows infection reduces translocation during the day, since it has been shown to have no effect during the night. As yellows infection increases the respiration rate of the leaves (van Riemsdijk, 1935) and decreases the ratio of area/residual dry matter (Table 20), infected leaves would

need to have a higher rate of photosynthesis per unit leaf area to produce the same gain in carbohydrate percentage of residual dry matter as healthy plants. Thus, the evidence on diurnal fluctuation of carbohydrate content of the leaves is contrary to the hypothesis that the reduced N.A.R. of yellows-infected plants is attributable to a greatly decreased rate of photosynthesis.

Determinations of chlorophyll content made on comparable leaves of healthy and yellows-infected plants grown in the field or in the glasshouse have shown that yellowed parts of leaves of infected plants are not devoid of chlorophyll, but contain 40–50% of the concentration present in healthy leaves. This difference appears insufficient in itself greatly to decrease the rate of photosynthesis, for Willstätter & Stoll (1918) showed that much wider variations in chlorophyll content had little effect, and though yellows infection may affect other parts of the photosynthetic mechanism than the chlorophyll, it is evidently unsafe to assume that the yellowing of the leaves implies a deterioration in photosynthetic activity.

It remains to consider the possibility that the decreased N.A.R. of yellows-infected plants was the result of increased respiratory loss of dry matter. Thomas & Hill (1949) found that the total respiration during the period from May to November of sugar-beet plants grown in large-scale sand-culture with external conditions similar to those of a field crop amounted to about 30% of the total photosynthesis, of which 20% was due to respiration of the tops and 10% to respiration of the roots, so that the net assimilation was 70% of total photosynthesis. The reduction of mean N.A.R. by yellows infection ranged between 16 and 48% (Table 12), ignoring the late infection in Exp. 1 which gave uncertain results. According to the data of Thomas & Hill, a reduction of 16% in N.A.R. brought about solely by increased respiration would correspond to a decrease in net assimilation from 70 to 59% of total photosynthesis, and would therefore require total respiration to increase from 30 to 41% of total photosynthesis, that is to say, the respiration rate of the whole plant would have to increase by nearly 40%. Similarly, to reduce N.A.R. by 48%, the respiration rate would have to increase by 110%. Van Riemsdijk (1935) found that the respiration rate per g. dry weight of yellows-infected sugar-beet leaves is 15–25% greater than that of comparable healthy leaves. An increase of this order in the respiration rate of the whole plant would obviously be far too small to account for the observed decrease in N.A.R. Assuming a 25% increase in respiration rate of the tops, and the relative magnitudes of top and root respiration given by Thomas & Hill, an increase of 60% in the rate of respiration of the root would be required to reduce N.A.R. by 16%, and a nearly fourfold increase to reduce N.A.R. by 48%.

The evidence is inadequate to decide whether the depression of N.A.R. by yellows infection results from change in photosynthesis or respiration, or both, but appears to be against any appreciable decline in the rate of photosynthesis, and suggests that the reduction of N.A.R. arises mainly from a large increase in the rate of respiration, especially of the root. The question can be settled only by direct

measurement of the rates of photosynthesis and of respiration of healthy and infected plants.

The most outstanding change in the composition of the plant induced by yellows was the large increase in reducing sugar content of the leaves, accompanied by smaller and less persistent increases in starch and sucrose content. Quanjer (1934) ascribed the starch accumulation in yellows-infected leaves to inhibition of translocation caused by blockage of the sieve-tubes of the phloem in the veins of the leaf and in the petiole by gummosis. However, it is doubtful whether phloem gummosis is a specific symptom of yellows infection, and there appears to be no close correlation between its occurrence and the onset of carbohydrate accumulation. Also, Watson & Watson (1951) showed that the loss of carbohydrate during the night from infected leaves is as great as from comparable healthy leaves.

A rise in carbohydrate content of the leaf without change in the rate of translocation could result from an increase in the resistance to movement of assimilate out of the leaf lamina. Assuming that carbohydrate is transported along gradients of sugar concentration by a process analogous to diffusion, the effect of increased resistance in the path of movement would be to steepen the gradient. The sugar concentration in the leaf would rise until a sufficient head developed to move sugar out of the leaf at a rate balancing its rate of production. If the rate of photosynthesis were unaltered, the rate of translocation would be the same after adjustment to the increased resistance as it was before. During the period of adjustment, the total translocation would be reduced by the extra carbohydrate retained in the leaf, but the change in the rate of translocation would be small if the increase in resistance developed slowly over a long period, because the additional carbohydrate accumulated in the leaf would be only a small fraction of the total translocation. For example, in Exp. 2 at sampling 2 the weight per sample of carbohydrate in the leaves of yellows-infected plants was about 40 g. more than in healthy plants, on the average of the two nitrogen treatments; the increase in root weight per sample in the interval between samplings 1 and 2, which is a minimal estimate of total translocation during the period when the rise of carbohydrate content developed, was about 350 g. Similarly, in the interval between samplings 2 and 4 there was a further increase of 50 g. per sample in the carbohydrate accumulated in infected leaves above that in healthy leaves, while the increase in root weight per sample of infected plants was 950 g.

If the resistance to movement out of the leaf is increased, carbohydrate should accumulate in yellows-infected leaves in the form in which it is translocated. The fact that the main increase is in reducing sugars agrees with the suggestion of Mason & Maskell (1928) that carbohydrate passes from the leaf mesophyll into the veins in this form. Phillis & Mason (1933), however, rejected this view, mainly because there is a much larger diurnal fluctuation of sucrose than of reducing sugars in the leaf lamina, and concluded that carbohydrate travels from the

assimilating cells of the leaf to the phloem as sucrose, but the question remains undecided.

Another possibility that could reconcile carbohydrate accumulation in infected leaves with an unchanged translocation rate is that the excess carbohydrate may be isolated in the assimilating cells so that it can play no part in translocation, and that the normal carbohydrate traffic, proceeding at the same rate as in healthy leaves, is superimposed on a static concentration of carbohydrate. No mechanism that could bring about such a change can be suggested. It might be possible to discriminate between this and the 'increased resistance' hypothesis by determining whether or not the difference in carbohydrate content between healthy and infected leaves persists after the plants have been held in darkness for a long period.

At first sight, the increase in the ratio of leaf dry weight/total dry weight (Table 8) and the reduction in root dry weight (Figs. 1 and 2) caused by yellows infection might be taken as evidence of decreased translocation rate, conflicting with the findings of Watson & Watson (1951). However, both may partly result from increased root respiration, and the lower root weight partly from reduced total translocation brought about by the decrease in total leaf area, not necessarily involving a change in the rate of translocation as percentage of residual dry matter of the leaf lamina.

Definite effects of mosaic infection on yield in Exp. 2 were established only for plants that received nitrogenous fertilizer. The growth changes responsible for loss of dry matter, though smaller, followed the same pattern as those caused by yellows infection; mosaic and yellows viruses both reduced total leaf area by restricting leaf expansion, and neither decreased leaf number; both caused about twice as great a percentage reduction in N.A.R. as in leaf area. This suggests that both affect growth processes similarly; if this is so, carbohydrate accumulation and yellowing in yellows-infected leaves are independent of the changes that affect dry-matter accumulation, because they do not occur in mosaic-infected plants.

It is not known whether the reduction of N.A.R. by mosaic infection derives from decreased photosynthesis or increased respiration. In growth-analysis studies, variation in N.A.R. is usually taken to indicate change in the rate of photosynthesis, but it would be unwise to assume that this is true for the effect of mosaic infection, in view of the uncertainty about the corresponding effect of yellows.

Little quantitative information on the effects of other virus diseases on the growth of their host plants has been published. Grieve (1943) studied the effect of tomato spotted wilt virus on the growth of young tomato plants in a period of about 1 month after inoculation, and found that after the appearance of leaf symptoms infected plants had both a smaller leaf area and a lower N.A.R. than healthy plants. The effect on N.A.R. was relatively greater than that on leaf area, and both were more severe when leaves were bronzed than when they showed only mottling. Infection with spotted wilt virus also reduced growth in height of the stem, and Grieve attributed this to destruction of auxin; he found that infected leaves

contained less auxin than comparable healthy leaves, and that when β -indole acetic acid was applied at the apical end of stem sections, less was recovered at the basal end in sections from infected plants than in sections from healthy plants. There is no stem extension in sugar-beet plants in their first year of growth, and the only effects of virus infection in the present experiments that could be taken as evidence of changed auxin supply are the reduction of leaf size by both yellows and mosaic infection, and the increased production of leaves from lateral buds in yellows-infected plants. Auxin causes the midrib and lateral veins to elongate, though it apparently does not affect expansion of the mesophyll (Went & Thimann, 1937). The increased development of lateral buds in yellows-infected plants suggests a partial breakdown of apical dominance, which could result from a reduction of auxin transport from the stem apex.

Bald & Hutton (1950) showed that potato plants grown from tubers infected with leaf-roll virus had a smaller leaf area than healthy plants, and concluded that the reduction of leaf area was sufficient to account for the loss of yield of tubers. They say 'it is possible, even, that under some circumstances diseased plants are more efficient per unit area of leaf than healthy plants in the production of tubers'. Presumably, this implies that the rate of dry-matter production per unit leaf area was either not affected or was increased by infection, but no data on N.A.R. are given to support this conclusion.

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THE SPREAD OF BEET YELLOWS AND BEET MOSAIC VIRUSES IN THE SUGAR-BEET ROOT CROP

II. THE EFFECTS OF APHID NUMBERS ON DISEASE INCIDENCE

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(With 4 Text-figures)

Multiple regression analysis of the data described in a previous paper identified alate *Myzus persicae* of the spring and summer migrations as the most important factor affecting spread of beet yellows virus in the sugar-beet root crop in England.

Apteræ of *M. persicae* spread little virus, and the contribution of alatae and apteræ of *Aphis fabae* was negligible.

A simple mathematical model of the spread of infection was developed. Assuming that the crop is visited by N aphids at a time when the proportion of plants infected is k_0 , the predicted proportion of infection for a time 3-4 weeks later (k_1) is

$$k_1 = k_0 + 100(1 - k_0)(1 - e^{-NI}),$$

where

$$I = p[(1 - k_0)^t + k_0 t - 1]/k_0.$$

This formula adequately accounts for the observed spread of infection when $N = 1/10$ sticky trap count for the 3-4 weeks preceding the time when k_0 infection is observed; p = probability of infection by a single aphid = $\frac{1}{2}$; t = number of movements per aphid effective for spreading beet yellows virus = 5.

The good fit of this formula to the observed data supports the results of the regression analyses in showing that alate *M. persicae* were mainly responsible for spreading beet yellows virus. Once infection had been introduced into the root crops in the spring spread was mainly within fields, or between fields in the same neighbourhood.

In areas where beet and mangold seed crops were grown intensively some infection was spread to the root crops by summer migrants from infected seed crops. Apart from this, the greater prevalence of yellows in seed crop areas was caused by the greater development of *M. persicae* infestation of the root crops which occurs in these areas.

The proportion of plants infected by spring migrants entering the root crops before the end of June was small and variable. It did not vary significantly between seed and non-seed areas. This implies either that initial infection came equally from sources other than seed crops, or that, if the seed crops were the main sources of infection, winged aphids acquired infectivity by visiting them during migration, and later became very widely dispersed.

In contrast to yellows, mosaic virus spread mainly in the neighbourhood of seed crops within the seed-crop areas. No significant relation between aphid numbers and increase of infection was established, but there is a strong suggestion that alatae of *M. persicae* and *A. fabae* spread the virus, and that the contributions of both species are equal. This suggests that mosaic virus is not spread by movement of aphids within the root crops, but only by infective migrants coming from

outside sources. As the virus is non-persistent the sources must be near to the root crops, and as there is little internal spread many infective migrants are needed to cause a high level of infection. The seed crops fulfil these conditions as they are often severely infected with mosaic, and both vector species breed on them. The irregularity of the data relating aphid numbers to mosaic incidence is probably caused mainly by individual variation in the intensity of infection of seed crops, and in the proportion of migrants caught on the traps which actually derive from them.

The first paper on this subject (Watson, Hull, Blencowe & Hamlyn, 1951) described a survey of sugar-beet fields in eastern and central England made between 1943 and 1948. The incidence of beet yellows and beet mosaic viruses was recorded throughout the growing season; *Myzus persicae* (Sulz.) and *Aphis fabae* (Scop.) populations were estimated by sampling and sticky traps were erected to provide estimates of winged aphid numbers. The results indicated that *M. persicae* is mainly responsible for the spread of beet yellows virus and that it also plays some part in the spread of beet mosaic. Both viruses were more prevalent in districts where beet or mangold seed crops were grown; *M. persicae* was also more abundant in these areas. In the present paper an attempt is made to investigate more quantitatively the main factors concerned, and to determine how much of the field to field variation in the incidence of the virus diseases they can account for.

DATA AND METHOD OF TREATMENT

As described previously (Watson *et al.* 1951), observations of aphid numbers and disease incidence were taken on a total of 103 fields over a period of 6 years. The fields were selected for their position relative to beet and mangold seed crops so as to belong approximately to one of the following four groups:

O_o	Outside the seed-crop areas	More than 1 mile from an isolated seed crop
O_s		Less than 50 yd. from an isolated seed crop
S_o	Inside a seed-crop area	More than 1 mile from a seed crop
S_s		Less than 50 yd. from a seed crop

The seed crop areas were determined by plotting the acreage of seed per parish on maps with a grid of 4×6 miles. Those sections of the grid with an average of more than 10 acres of seed crop per 24 sq.miles were defined as seed-crop areas.

A more quantitative measure of seed-crop concentration was given by the total acreage of seed crop in an area of 216 sq.miles formed by the nine sections of the map grid surrounding each field. The seed-crop data were not available for all years, so the 1944 figures were used for 1943 and 1944, the 1946 figures for 1945 and 1946, and the 1947 figures for 1947 and 1948. There were no important changes in the main concentrations of seed crop over the 6 years.

The ways in which the data were obtained have been described in the previous paper. We refer to the numbers of alate aphids per sq.ft. of sticky trap as *trap-counts*

and to the numbers of adult apterae counted on 100 plants as *field-counts*. Counts obtained throughout the season may be simply added to obtain total trap- and field-counts; they may also be combined in a way that gives greater importance to the early migrants whose contribution to the spread of infection was expected to be large by analogy with results obtained with potato viruses (Davies, 1934). In detail, the sum is taken of

total catch up to 16 June	× 5
17-30 June	× 4
1-16 July	× 3
17-31 July	× 2
1-31 August	× 1

and the resulting total gives a count expressed as *aphid infestation fortnights* (A.I.F.).

The infection counts were not all taken on the same series of dates throughout each season. The figures used in the analyses all refer to the third week of September, and were obtained by a rough graphical interpolation or extrapolation where required. This date was reasonably appropriate for aphid counts taken up to the end of August as symptoms take 3 or 4 weeks to develop.

For the regression calculations below, the aphid- and seed-crop figures used as independent variates were transformed to logarithms (cf. Williams, 1937). In order to stabilize the variance of the infection figures used as dependent variates, the observed percentages were transformed to degrees (Fisher & Yates, 1948, Table XII). These two conversions rendered the relation between infection (measured in degrees) and log total trap-count roughly linear, as can be seen from Fig. 1; unless otherwise stated, they are used throughout the paper. As interest centred on field to field rather than on overall year to year comparisons, differences between years were eliminated in the course of the analysis.

Most of the investigation consisted of the calculation of linear regression equations of the infection figures (in degrees) on various independent variates representing the factors thought to be of importance. The object of these calculations was to discover the amount of the variation in infection level accounted for by the various factors rather than to provide actual prediction equations which would in any case change from year to year; consequently, the presentation gives simply the regression coefficients with their standard errors, the residual standard deviation of the dependent variate and the proportion of the total variance accounted for by the regression (V.A.R.).

Myzus persicae and *Aphis fabae* as vectors of virus yellows

To compare the two species of aphid as vectors of virus yellows, a multiple regression equation was fitted in which the infection level with virus yellows, measured in degrees, was related to the A.I.F. trap counts of *M. persicae* (x_1) and of

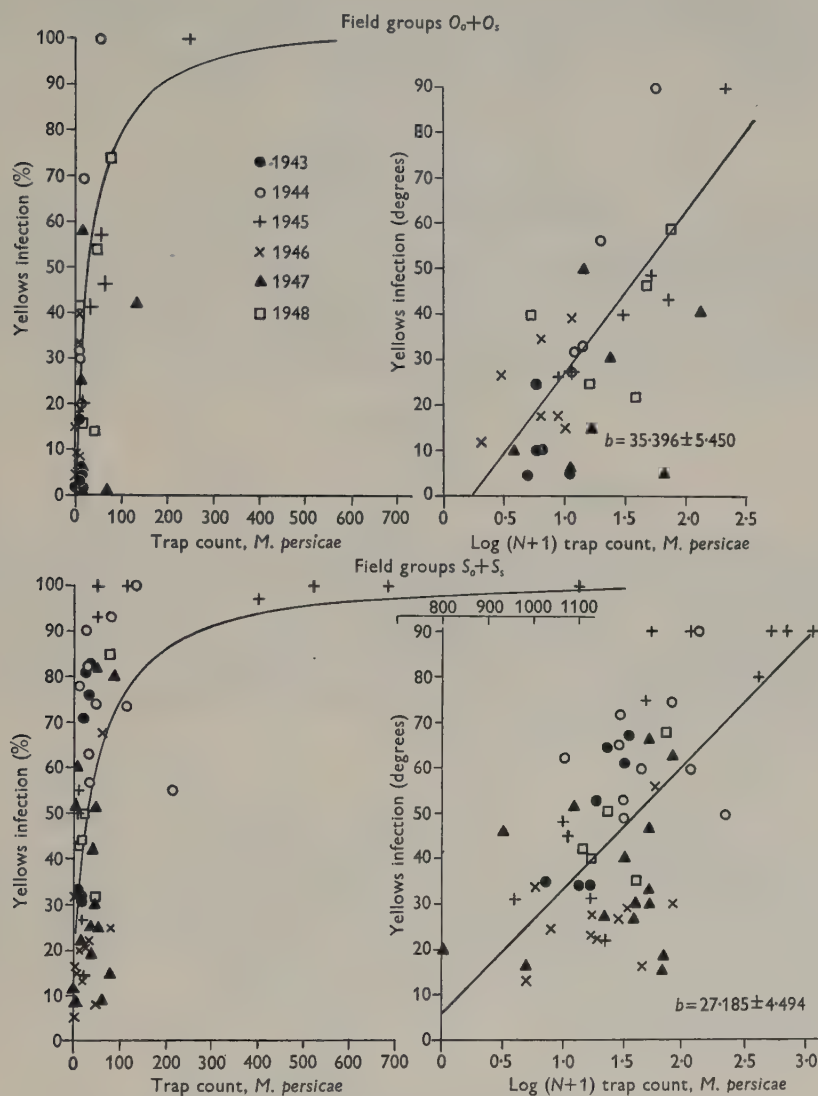


Fig. 1. The relation between percentage of plants infected with beet yellows virus in the third week of September, and total trap-count up to the end of August. The top two figures show fields out of, and the bottom two figures fields within, the seed-crop areas. The left-hand figures show percentage infection plotted against arithmetic totals for trap-counts, and the right-hand figures show degrees of infection plotted against $\log (N+1)$, total trap-counts. The curves on the left-hand figures are reconversions of the regression lines plotted on the right-hand figures.

A. fabae (x_3) and to the total field counts of the two species (x_2, x_4), all measured logarithmically. The regression coefficients for y on x_1, x_2, x_3 and x_4 (b_1, b_2, b_3, b_4), are as follows:

$$\left. \begin{aligned} b_1 &= +22.88 \pm 4.70, \\ b_2 &= +4.00 \pm 2.84, \\ b_3 &= +3.04 \pm 3.95, \\ b_4 &= +0.36 \pm 2.58, \\ \text{residual S.D.} &= 15.0^\circ, \quad \text{V.A.R. } 40.0\% \end{aligned} \right\} \quad (1)$$

The alate *M. persicae* measured by the trap count provide the dominant term. Independently of this, apterous *M. persicae* measured by the field count may have given some effect, but the two coefficients relating to *A. fabae* are small compared with their standard errors. If these two terms are eliminated, the results are:

$$\left. \begin{aligned} b_1 &= +22.42 \pm 4.13, \\ b_2 &= +4.33 \pm 2.62, \\ \text{residual S.D.} &= \pm 14.9^\circ, \quad \text{V.A.R. } 40.8\% \end{aligned} \right\} \quad (2)$$

The fit is slightly better than before, and the size of the apterous term has increased somewhat. It will be shown later that the expression of the trap-counts as aphid infection fortnights does not do full justice to the distribution of the counts throughout the season, and it may be that the term in x_2 really reflects this; for a fixed trap-count, a high field count is usually associated with a larger number of winged aphids early in the season, so that inclusion of the field count term may merely give extra weight to the trap-count.

The effect of nearby seed crops on the spread of virus yellows

The first paper showed that virus yellows was more prevalent in seed-crop areas, but it was suggested that this might have been due to the larger number of aphids in these areas. To investigate this point, a new variate was added to the two-term regression (2) above. This term (x_5) is given by the acreage of seed crop in the surrounding area of 216 sq.miles expressed as a logarithm.

The results were as follows:

$$\left. \begin{aligned} b_1 &= +20.07 \pm 4.24, \\ b_2 &= +5.04 \pm 2.69, \\ b_5 &= +5.74 \pm 2.99, \\ \text{residual S.D.} &= \pm 14.6^\circ, \quad \text{V.A.R. } 42.7\% \end{aligned} \right\} \quad (3)$$

It appears that, even after allowing for the different aphid populations, infection is slightly increased in areas where seed crops are common.

It was thought that the presence of seed crops might alter the relation between infection and aphid numbers. To test this, a product term ($x_1 x_5$) was added to the

equation. The resulting coefficient was small and negative, implying that the increase in infection due to a ten-fold increase in aphid numbers was, if anything, less when the seed-crop acreage was high, contrary to expectation. It appeared, therefore, that this type of interdependence was unlikely on the present evidence.

The spread of yellows virus throughout the season

Fig. 2 shows the way in which the level of infection with yellows virus increased throughout the growing season at four typical centres. In general, the curves show a slow increase in infection up to mid-July followed by a rapid rise to a

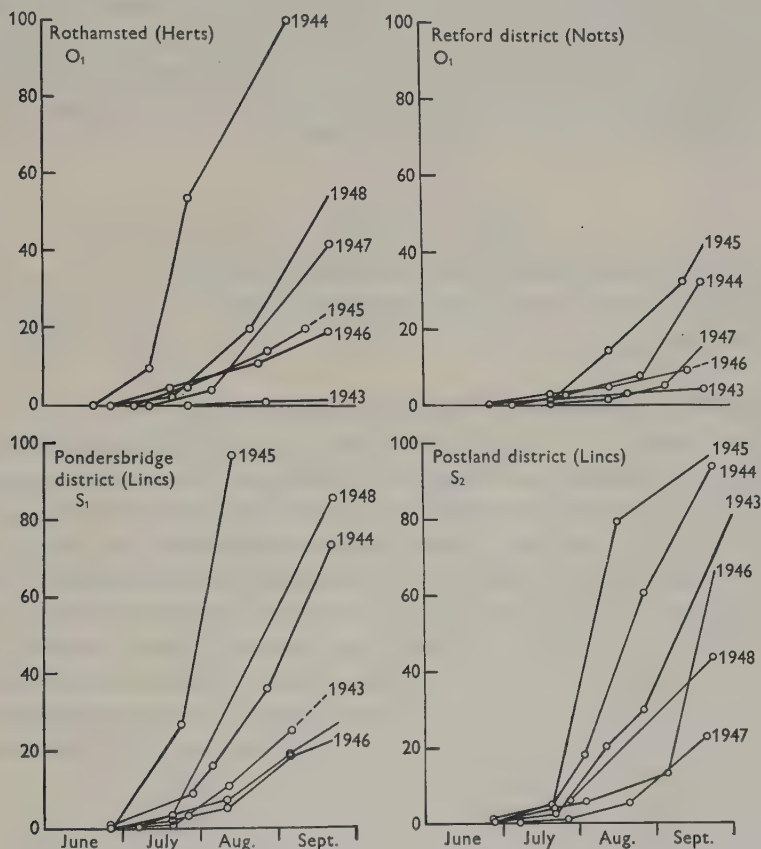


Fig. 2. The increase of percentage of plants infected with beet yellows virus throughout the season at four centres for 6 successive years.

maximum in September or October, but in some fields the increase is rapid from June or early July (e.g. Rothamsted, 1944; Pondersbridge, 1945.) The regression analysis has shown that the numbers of winged *M. persicae* have the greatest effect

on the spread of yellows disease, this effect being modified to some extent by the area of seed crop in the neighbourhood. The spring migration of *M. persicae* into the root crop is completed before the main rise in infection occurs in most fields, and it is plausible to suppose that overwintering sources of infection affect the final levels mainly through their influence on the amount of disease brought into the root crop at the beginning of the season, the main increase in infection being due to spread within the root crop.

With these considerations in mind, a very simple mathematical model of the spread of infection was developed. We suppose that a field contains a proportion of infected plants. An aphid visits successively t plants at random. If one of the plants visited is infected with virus, the aphid becomes *infective*, and thereafter has a probability p of infecting the subsequent plants that it visits. On these assumptions, it can be shown that if the crop is visited by N aphids per plant at a time when the proportion of plants infected is k_0 , the predicted proportion infected afterwards is

$$k_1 = k_0 + (1 - k_0)(1 - e^{-NI}),$$

where

$$I = p[(1 - k_0)^t + k_0 t - 1]/k_0.$$

The derivation of this formula is given in the Appendix.

To apply the formula to the data, numerical values of the different parameters had to be settled.

(1) *The number of aphids visiting the crop in a given period*

Since total numbers of winged aphids over a period are required, and field-counts give only instantaneous values, the trap-count data were more appropriate for use with the formula. However, it was necessary to find some way of relating the trap-counts to the number of alatae which actually visit the plants. This had already been attempted by other workers for comparable data obtained from potato crops. Doncaster & Gregory (1948), and Broadbent, Chaudhuri & Kapicza (1950), described experiments in which sticky traps similar to those used by us were exposed near to sets of potted potato plants, and daily records kept of the alate *M. persicae* caught on the traps and counted on the plants. In two series of experiments made during 1944 and 1945, the number of aphids counted on fifty plants averaged about $4\frac{1}{2}$ times the sticky trap count.

In a different series of experiments, Broadbent (1948) compared the catches of *M. persicae* on traps set at 6 ft. from the ground (as ours were), and others set at plant level among a potato crop. The average ratio between trap counts was 13:1.

Although these results were obtained with potatoes it seemed likely that they were applicable to other crops, and it was assumed that the number of aphids per plant which visit a sugar-beet crop (N) could adequately be estimated as one-tenth of the trap-count.

(2) *The probability of infection by a single aphid*

The proportion of plants infected by single individuals of *M. persicae*, given a single infection feeding with yellows virus and tested on successions of healthy seedlings, varies with experimental conditions and with the strain of virus used. In the experiment described on p. 56, made with a strain of moderate virulence, it was 41%, but strains of much higher transmissibility have been isolated from the field. For the purpose of the calculation the average probability of infection by a single aphid which has had adequate feeding time on infected and healthy plants was taken as $P = \frac{1}{2}$.

The probability of infection decreases with increasing age of the receptor plant, and might be expected to be smaller in July and August than in June, but for the later times there is increased possibility of the aphids feeding on more than one infected plant during their five effective moves; these two effects tend to cancel each other out, and P has been kept constant throughout the calculation.

(3) *The number of effective moves made by alatae*

Although there is evidence that alate *M. persicae* move about in a sugar-beet crop and visit several plants, there is little data on which to base an estimate of the number of moves made. Accordingly, values of t ranging from 3 to 9 were tried in the formula. Comparison with the field data showed that the best fit was given by $t = 5$.

An average of five effective moves per alate *M. persicae* seems reasonable, for nymphs produced by *M. persicae* spring migrants are usually found in groups of one to three individuals. In the glasshouse alatae produce from ten to twenty nymphs per adult, depending on conditions. This suggests about five to ten moves per adult, but there would be some loss by early mortality in field conditions and other contingencies, leaving about the five already suggested by the trial and error method. An effective move is defined as one which involves at least 3–4 hr. of feeding, because beet yellows virus is inefficiently transmitted in shorter feeding times.

Summarizing, we use the following values:

number of alatae visiting each plant, $N = \frac{1}{10} \times \text{trap-count}$;

number of effective visits per aphid, $t = 5$;

probability of infection after a visit by an infective aphid, $p = \frac{1}{2}$.

These figures are only approximate, and it was intended to vary them so as to obtain the best possible fit to the data. However, when used in the prediction formula they gave such good agreement with the observed infection figures that it was not thought worth while to make any further adjustments.

In the field, symptoms of yellows disease take about 3 weeks to develop in young plants early in the season, and 4 or more weeks on older plants, or in less favourable

conditions. Observations were made at 3-weekly intervals during June and July and at rather longer intervals later on, so that plants recorded as infected at one observation date would have received the virus during a period ending at about the time of the previous observation date. For example, plants showing infection on 8 July would have received it from aphids active between about 24 May and 16 June, and it is to the trap-counts over this period that the increase in infection must be related.

The way the formula was applied can be illustrated by an example, Table 1.

TABLE 1. *Observed and predicted percentage infection with yellows virus at Buckden, Hunts, 1944 (S_0 field)*

8-day periods*...	June				July				August				September		
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
Trap-count	—	1	—	2	—	—	17	—	9	—	—	0	—	—	0
% infection:															
Observed	—	0	—	1.2	—	—	4.3	—	16	—	—	41	—	—	82
Predicted	—	—	—	—	—	—	3.0	—	—	24	—	—	—	67	—

* See Doncaster & Gregory (1948), Watson *et al.* (1951).

For this field the trap-count for the last 3 weeks of June was three aphids, so that putting $k_0 = 0.012$, $N = 0.3$, the formula is found to give $k_1 = 0.030$, compared with an observed figure of 0.043. The trap-count for the first 3 weeks of July was seventeen, so that (using the *predicted* infection level) we put $k_0 = 0.030$, $N = 1.7$ and obtain $k_1 = 0.24$, while a further period with $N = 0.9$ gives $k_1 = 0.67$.

This example is shown graphically in Fig. 3 (*a*). It illustrates what may be accepted as a typical curve of increasing infection. The number of spring migrants was about average, and there was a moderate peak of infestation in the third week of July. Fig. 3 (*b*), (Rothamsted, 1944) shows the result of numerous spring migrants in May and June, causing early summer infestation; the observed level of infection has risen almost linearly. Fig. 3 (*c*) illustrates the result of the small spring migration and late summer infestation of 1947, after a very severe winter. The level of infection remains low up to the end of August, but is abruptly increased by an early autumn infestation. Fig. 3 (*d*) is characteristic of 1946 when, after a moderate spring migration, cold weather in June and July prevented the infestation from developing, and little virus infection appeared in the crops. In these four instances the predicted figures, based on the earliest recorded infection and the trap-counts, follow the observations quite well. The other two examples show discrepancies. At Buckden in 1945 the observed figures were a good deal larger than the predicted; this may have been due to a badly sited trap failing to catch a representative number of aphids. At Spalding in 1946 the predicted rise in infection during August failed to materialize. The trap was sited between the observation field and a neighbouring

field of cabbages, and many of the aphids caught may have belonged to the brassica crop and not to the sugar beet.

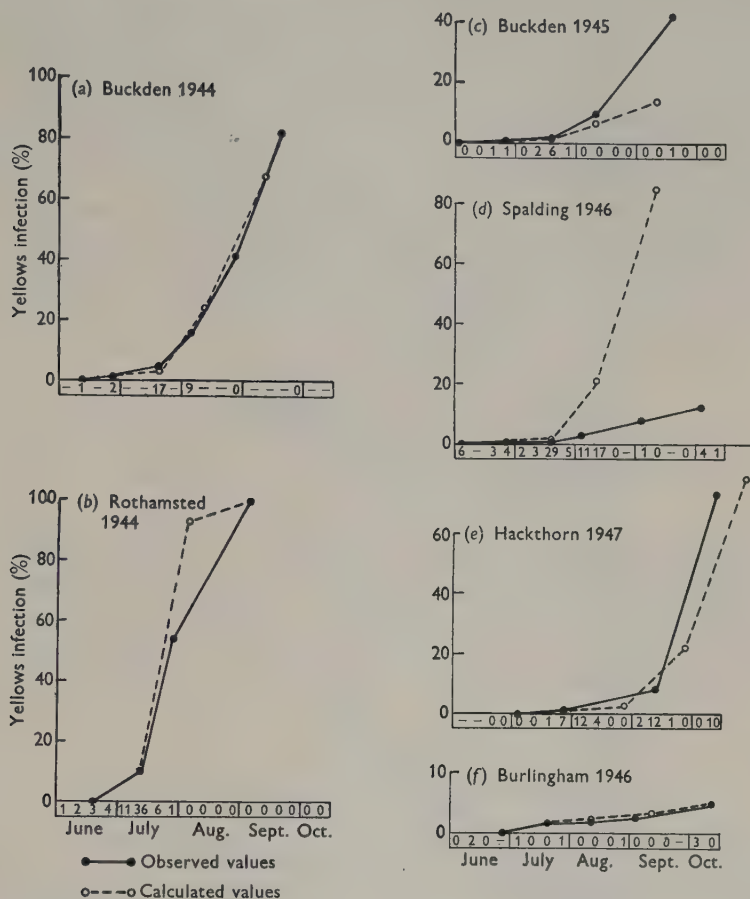


Fig. 3. Examples of fitting calculated to observed values for percentage infection throughout the season. The abscissae are divided into boxes each representing 1 month, and the four figures within them represent the weekly trap-count. In (a) the traps were cleared on the observation dates. For other fields they were cleared weekly, or occasionally the same trap was left for 2 successive weeks, the first being denoted by a dash, —. The broken line, representing the calculated values, starts at the first adequate recorded infection, the previous count having been 0.1 % or zero.

By rough graphical interpolation observed and predicted infection levels were obtained for the second week in August and the third week in September. After conversion to degrees these are shown plotted in Fig. 4.

The predicted infection level may be thought of as a transformation of the trap-count figures with the same purpose as the use of aphid infestation fortnights. We can thus use the predicted levels as an independent variate in regression

calculations. Taking the September figures, and omitting the two aberrant values mentioned above, and two others (Hackthorn, 1944; Terrington, 1947), for which

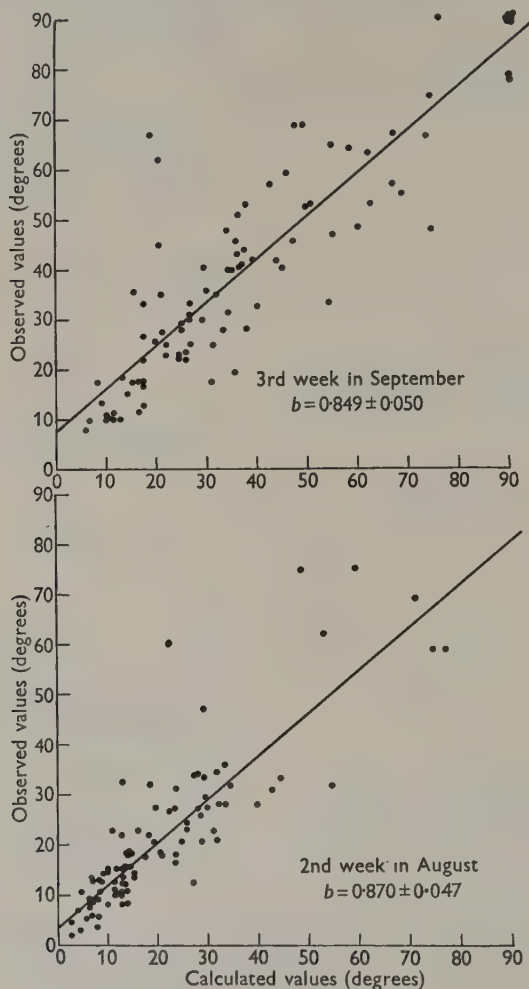


Fig. 4. Relation between observed and calculated values for degrees of infection with beet yellows virus in the third week of September and the second week of August. The regression calculation includes the aberrant values mentioned above, except Hackthorn, 1944.

the trap-counts were known to be faulty, regression of infection level in degrees on the predicted level also converted to degrees (x_6) gives the following results:

$$b_6 = +0.907 \pm 0.046, \text{ residual S.D.} = \pm 8.1^\circ, \text{ V.A.R. } 82.8\%. \quad (4)$$

Although the regression coefficient is well below its expected value of 1, the fit is remarkably good considering the nature of the data, the simplicity of the mathe-

mathematical model, and the relatively crude values adopted for the different parameters. By varying these values, an even better fit could certainly be obtained, although the improvement would probably not be very great. The regression coefficient is less than unity largely because of errors in the independent variate. This is based on an early infection count (taken when the infection level was often less than 1%) and on the trap-counts throughout the season. Neither of these measurements can be considered accurate—the presence or absence of a single aphid often makes a substantial difference to the final result—and some depression of the regression coefficient is to be expected. A further contributory factor is the interpolation needed to bring all the results to the same date, which is necessarily rather rough.

When independent variates based on total field count of *M. persicae* (x_2) and seed crop acreage (x_5) were added the results were as follows

$$\left. \begin{aligned} b_2 &= +1.47 \pm 1.33, \\ b_5 &= +4.14 \pm 1.58, \\ b_6 &= +0.856 \pm 0.051, \\ \text{residual S.D.} &= \pm 7.9^\circ, \quad \text{V.A.R. } 83.9\%. \end{aligned} \right\} \quad (5)$$

The apterous aphids are of doubtful importance when the other two factors are held constant, but there is still more infection in areas of high seed-crop acreage, presumably because some of the summer migrants in these areas are already infective when they arrive on the root crop. If the term relating to apterae is removed, the results become

$$\left. \begin{aligned} b_5 &= +3.99 \pm 1.57, \\ b_6 &= +0.884 \pm 0.045, \\ \text{residual S.D.} &= \pm 7.9^\circ, \quad \text{V.A.R. } 83.9\%. \end{aligned} \right\} \quad (6)$$

In summary, the investigation indicates that the predominant factor in the spread of virus yellows is the number of alate *M. persicae* present through the season. It appears that after infection reaches the crop initially the main spread takes place within the fields, the aphids carrying the virus from infected to healthy plants. However, the significance of the seed-crop term in equations (5) and (6) may be taken to indicate that some virus is brought in from outside the crop during the whole of the season, especially in regions with a large seed-crop acreage. About four-fifths of the total variation between fields in the same year has been accounted for by the regression equations; improved values for the constants in the prediction formula might slightly increase this proportion, but the nature of the data is such that no great increase could be expected, and it appears unlikely that any factor of major importance relative to spread after initial introduction of infection has been overlooked.

Initial infection

After initial infection, the number of winged aphids, with a small contribution from seed crops, accounts for almost all the variation in subsequent spread of infection. In most years the first week of July may be taken to be the time at which all plants infected by spring migrants from outside sources will show symptoms, but in 1947 the spring migration was very late, and initial infection did not show until the third week of July.

A multiple regression equation was fitted in which the initial infection level, (y), was related to actual numbers of *M. persicae* trapped up to the end of June (2nd week of July for 1947), (x_1), and log acreage of seed crop per 216 sq. miles, (x_2). Where there was no observation in the first week of July, the percentage infection was calculated on the assumption that increase between June and July is linear. The regression coefficients of y on x_1 and x_2 are as follows:

$$b_1 = 0.0532 \pm 0.0149,$$

$$b_2 = 0.1411 \pm 0.1643.$$

$$\text{V.A.R.} = 12.7\%.$$

These results show that initial infection is related to the size of the spring migration of *M. persicae*, but there is no significant relationship with seed-crop distribution. The great variability between fields in both virus and aphid counts at this time of the year is reflected in the size of the errors, and the small proportion of variance which is accounted for by the regression.

Factors influencing the spread of beet mosaic diseases

The first paper showed that the incidence of mosaic was connected with the seed-crop acreage near the sampled fields. This can be seen from Table 2 which gives the mean percentage infection with mosaic in the four field-groups in the 6 years. The observations were expressed in degrees and the means converted back to percentages.

TABLE 2. *Mean percentage of plants infected with mosaic virus in the four field groups for the years 1943-48*

	Non-seed area (O)		Seed area (S)	
	<i>o</i>	<i>s</i>	<i>o</i>	<i>s</i>
1943	0.0 (5)	— (0)	2.2 (4)	32.2 (3)
1944	1.8 (4)	1.8 (1)	11.5 (5)	35.0 (5)
1945	0.4 (5)	21.7 (4)	2.2 (5)	20.9 (5)
1946	0.1 (5)	0.6 (3)	0.2 (7)	2.5 (5)
1947	0.1 (5)	0.1 (1)	0.4 (7)	1.7 (6)
1948	0.1 (2)	0.1 (1)	1.2 (2)	6.5 (3)

s = within 50 yd. of a seed crop.

The figures in brackets show the number of fields in each group.

It is clear that appreciable attacks of mosaic were almost confined to fields in a seed area that were close to a seed-crop. The results in the O_s fields are few and irregular; the high mean for 1945 includes the only field with 100% infection.

The regression technique cannot extract useful information from field-groups other than group S_s , because there is in general no variation to explain. A four-term regression on the variates representing trap and field counts of *M. persicae*, (x_1 , x_2), and *A. fabae*, (x_3 , x_4) as in equation (1), was fitted to the figures in the s group only, with the following results:

$$\left. \begin{aligned} b_1 &= +30.42 \pm 16.98, \\ b_2 &= -9.53 \pm 8.78, \\ b_3 &= +36.63 \pm 21.31, \\ b_4 &= -0.26 \pm 5.76, \\ \text{residual S.D.} &= \pm 16.7^\circ, \quad \text{V.A.R. } 5.3\%. \end{aligned} \right\} \quad (7)$$

None of the terms is significant, but the trap-counts of both species predominate. Eliminating the field-count variables, we get

$$\begin{aligned} b_1 &= +14.52 \pm 9.77, \\ b_3 &= +29.46 \pm 17.25, \\ \text{residual S.D.} &= \pm 16.4^\circ, \quad \text{V.A.R. } 8.6\%. \end{aligned}$$

Again, neither the separate terms nor the regression as a whole reach the 5% level of significance, but it seems safe to assume that both species of aphid are concerned with the spread of mosaic, the alatae being of greater importance than the apterae.

Year-to-year variation in infection levels

So far, we have considered only differences between fields in the same year. In the first paper, the relationship between the yearly mean incidence of beet yellows and beet mosaic viruses and aphid numbers were shown diagrammatically, and statistical analysis adds little to the information thus presented.

The annual means of the transformed variates y (level of infection with yellows), x_2 (apterae per 100 plants), x_5 (seed-crop area) and x_6 (infection level predicted from early infection and numbers of alatae) are given in Table 3.

TABLE 3. Annual means for observed yellows infection, (y), apterous *M. persicae*, (x_2), seed-crop acreage, (x_5), and predicted infection level, (x_6). For explanation of y_{adj} , see text

	y	x_2	x_5	x_6	y_{adj}
1943	31.9	1.99	1.06	26.0	44.6
1944	58.4	2.57	1.20	54.7	44.9
1945	59.0	2.35	1.11	57.8	43.7
1946	21.6	1.39	1.04	20.9	39.6
1947	32.2	1.60	1.34	30.8	40.1
1948	40.0	1.24	1.36	38.5	41.8

The last column of the table, headed y_{adj} , gives the values of y adjusted to constant values of the x 's ($x_2 = 2.00$, $x_5 = 1.20$, $x_6 = 40.0$) by the within-year regression coefficients given at (5). Clearly, most of the variation between years is accounted for; a rough test of significance gives $\chi^2 = 6.7$ (5 D.F.).

Table 4 gives the annual means for mosaic infection, based on fields in S_s group only. The transformed variates are y (level of infection with mosaic), x_1 (A.I.F. trap-count *M. persicae*) and x_3 (A.I.F. trap-count *A. fabae*).

TABLE 4. Annual means for mosaic infection, (y), alate *M. persicae*, (x_1), and alate *A. fabae*, (x_3); S_s fields only

	y	x_1	x_3
1943	34.6	1.66	0.49
1944	36.2	2.15	1.53
1945	27.2	2.13	1.10
1946	9.1	1.43	1.42
1947	7.5	1.83	2.41
1948	14.9	1.80	1.59

There is no close relationship between the yearly mean values and the aphid figures. Possible reasons for these large differences in the effects of different factors on distribution of beet yellows and beet mosaic virus will be considered in the discussion.

DISCUSSION

Statistical analysis of the data described previously (Watson *et al.* 1951) indicates that beet yellows virus was spread mainly by alate *M. persicae*. Neither alatae nor apterae of *A. fabae* appeared to have any effect, and the independent contribution of apterous *M. persicae* counted on the plants was small. The failure of *A. fabae* and field-count *M. persicae* is presumably attributable to their sedentary habits. For *A. fabae* this hypothesis has become generally accepted, largely by analogy with the work of Doncaster & Gregory (1948) on *A. rhamni* in relation to potato viruses. Both species are irregularly dispersed in the crop, and migrant alatae apparently move infrequently from the plant on which they first alight.

It has generally been assumed that apterae of *M. persicae* contribute appreciably to spread of beet yellows virus. They are more or less evenly distributed throughout the crop, are easily disturbed and so are often seen moving about. These attributes have suggested for them a capacity to spread infection which seems not to be confirmed by their actual performance. Their random distribution probably reflects the activity of the colonizing migrants, and the proportion of the population which is moving at any time is likely to be small. Those individuals which do move probably cover only short distances, and cannot transmit infection unless they move from an infected to a healthy plant.

An experiment made in 1941 showed that yellows virus can be spread appreciably by apterae in some conditions. Plots on which varying proportions of plants

were deliberately infected at random were isolated from healthy control plots by muslin screens 22 in. high, which prevented contact between the leaves. Where 33% and upwards of the plants were experimentally infected the percentage of infection doubled in about 4 weeks within the screened plots, but did not spread from these to the control plots. This indicates that the spread was by apterae and not by alatae.

Obvious spread of infection by apterae occurred in this experiment, because initially there were many infected plants randomly distributed in each plot. In the observation fields the average infection up to the end of June was less than 1%. Few apterous *M. persicae* occur in the crop at this time, for only thirteen out of the 103 fields had more than one adult per plant before the second week of July. It seems impossible that these apterae, with their restricted movements, could build up the level of infection in such a way that the peak infestations of apterae alone could account for the observed spread.

To show the difference between apterae and alatae by the regression method is not easy, because their numbers are closely correlated. When single and multiple regressions were calculated for the data relating percentage infection to trap- and field-count totals obtained at the time of peak infestation, the coefficients were of about the same magnitude. Either alone accounted for about 35% of the variance, and both together for only about 40%. This showed that both counts were providing a measure of the same factor. When the multiple regression was calculated with weighted A.I.F. trap-count totals as one independent variate, and total field-count apterae up to the end of August as the other, the contribution of the apterae appeared small (p. 42), and when the trap-count data were combined with initial infection in the prediction formula, the observed spread of yellows virus was adequately accounted for, and the contribution of the apterae was negligible (p. 49). The assumptions on which the prediction formula is based are not applicable to apterae, since it cannot be assumed that all apterae move from plant to plant, that those which do will make several movements, or that the movements will be at random.

Spread within and between fields in the same district

The hypothesis from which the prediction formula was derived is that after introduction into the root crop in the spring beet yellows virus is mainly spread by random movements of alate *M. persicae* about the crops. The good fit achieved without any allowance for the subsequent introduction of infection by aphids indicates that the main spread of infection is 'internal', but this does not necessarily mean within single fields. In 1943 two fields were selected for observation in each neighbourhood, so as to provide comparisons within as well as between districts. The results for these pairs of fields were so similar that in later years the method was dropped because it was thought that the labour involved would be better employed in more extensive observations. So long as fields have similar levels of infection

there is no way of distinguishing spread within from spread between crops. There is evidence to show that migrants do move between crops in the summer, because infection is carried from the seed crops to the root crops. This is shown by the significant contribution of the seed-crop term in equations (5) and (6), p. 49. If there is exchange of migrants between the seed crop and the root crop there is no reason why it should not occur between root crops also.

The sources of initial infection

Initial infection is defined as the percentage of plants showing symptoms in the first week of July, except for 1947 when the third week of July was taken. It increases with increasing numbers of spring migrant *M. persicae* (p. 50), but there appears to be great variability between fields in the proportion of spring migrants which are infective. Crops frequently showed early infection when the number of aphids had been too small to be recorded on the sticky traps, but, on the other hand, a field on which thirty-six spring migrants were trapped, remained healthy.

The average trap-count of *M. persicae* up to the end of June was about four, which, on the assumptions made previously, represents about 0.4 alatae per plant in the crop. If all were infective they could infect about 60% of the plants, but the average initial infection is less than 1%, so that few aphids actually cause infection.

Initial infection in the seed crop was not significantly greater than in other areas, which suggests that the spring migrants were not produced on the seed crops. Up to 1949 a high proportion of seed plants were infected, and if aphids migrated directly from them to the root crops a higher level of initial infection in seed-crop areas would be expected and a greater difference between these and other areas.

M. persicae which overwinter on the steckling beds in mild winters, are usually killed by being buried when the seed crops are planted out in the spring. Re-infestation depends on spring migrants from other sources and takes place at the same time as initial infestation of the root crops. The seed crops, therefore, are breeding sources for infective migrants in the summer, but not in the spring. The effect of these summer infective migrants is seen in the contribution of the seed crops to the spread of infection which takes place after initial infection.

As beet yellows virus may persist for several days in both fasting and feeding vectors (Watson, 1946), migrants could still be infective after being carried on air currents many miles from the infected source. Therefore infection could be spread both near and far from seed crops, by aphids feeding on them temporarily during migration flights. If aphids do become infective during migration they are most likely to do so from the seed crops, which are far the largest exposed sources of infection during the critical periods in May and early June. On the other hand, clamped mangolds (Broadbent, Cornford, Hull & Tinsley, 1949), overwintering horticultural crops and perennial wild species of *Chenopodiaceae* are all possible sources of overwintering *M. persicae* and of the virus. These are presumably equally

distributed between the seed crop and other areas, and would be likely to provide the very irregular amounts but more or less even distribution of initial infection which is actually observed.

As initial infection appears not to be greatly affected by the proximity of seed crops, it must be assumed that the increased prevalence of yellows in seed-crop areas is due partly to the increased development of infestation with *M. persicae* on the root crops, and partly to infection being brought to them by summer migrants from the seed crops.

Spread of beet mosaic virus

The incidence of beet mosaic, unlike beet yellows, is not closely related to numbers of *M. persicae*, but it is very closely related to seed-crop distribution. Possible local sources of infection in the non-seed areas, mangolds, spinach, etc., are at least as susceptible to beet mosaic as they are to beet yellows virus, and it is difficult to see why yellows and not mosaic is spread in these areas if most of the migrants which introduce infection to the root crops come from these local sources. If both viruses come mainly from seed crops the lack of spread of beet mosaic in non-seed areas can be explained, because beet mosaic is a non-persistent virus and vectors rapidly lose their ability to cause infection after leaving the infected source.

Alatae of both *M. persicae* and *A. fabae* probably contribute to spread of beet mosaic virus, although neither gave a significant regression coefficient (p. 51), and the variance accounted for was very small. This difference between beet mosaic and beet yellows viruses resembles that between potato virus Y, which is non-persistent, and potato leaf-roll virus, which is persistent. Doncaster & Gregory (1948) and Broadbent (1950) have shown that though incidence of leaf-roll virus can be correlated with numbers of *M. persicae*, it is more difficult to show a similar correlation for potato virus Y. Hansen (1941) stated that the incidence of rugose mosaic in Denmark could be predicted from a combination of climatic and geographic factors which affect distribution and number of *M. persicae*, but he gave no measure of the significance of the direct relationship between spread of virus and aphid numbers.

With non-persistent viruses only very short feedings are necessary for vectors to become infective, and such short feedings might be made by migrants of species which do not breed on the crop, but may feed during migration while searching for their natural host. Dickson, Swift, Anderson & Middleton (1949) showed that cantaloupe mosaic virus is transmitted by migrant *M. persicae* in California, although *M. persicae* does not breed on cucurbit crops, but they record many more migrants than those of any species recorded in this country. In the present series of observations the willow-carrot aphid, *Cavariella aegopodii*, was the most numerous species in May and June, but it was very much more numerous in 1946 and 1947 when there was little spread of beet mosaic virus, than in 1945 when there was much.

Therefore there seems little suggestion that a large amount of variance in spread of beet mosaic which is not accounted for by *M. persicae* and *A. fabae* could be accounted for by other species.

Differences between beet yellows and beet mosaic viruses in relation to spread of infection

Beet mosaic, being non-persistent, is most readily transmitted by aphids which have fasted before a very short infection feed. Without fasting, or with longer infection feeding times, vector efficiency is much reduced. Even an optimally infective vector of beet mosaic can infect only the first plant it feeds on, unless it moves very rapidly from plant to plant so that the feeding times and intervals between feedings are not more than a few minutes. Probably this rarely happens except with non-breeding vector species mentioned above.

An experiment described previously (Watson, 1946) suggests that field conditions are more favourable for transmission of yellows than mosaic. *M. persicae* and *A. fabae* were fed on plants infected with both viruses together, and then for 10 min., 2 hr., and two periods of 24 hr., respectively, on successions of four healthy plants. After 24 hr. feeding on the infected plants yellows was transmitted by *M. persicae* to an average of 41% of all the healthy plants; mosaic was transmitted to 6%. Yellows was transmitted by *A. fabae* to 10% of the plants and mosaic to 3%. After preliminary fasting and 5 min. infection feeding no yellows was transmitted. Mosaic was transmitted by *M. persicae* to 50% of the first healthy plants, but the average for all feedings was only 14% for *M. persicae* and 1% for *A. fabae*.

Thus, in conditions where movement of aphids effectively spreads beet yellows virus, the chances of beet mosaic being spread are small; probably these conditions are the rule, because beet yellows is almost invariably transmitted where sufficient infective sources and numbers of aphids exist. The normal behaviour of migrant *M. persicae* seems to be better adapted to transmission of beet yellows virus, and it may be that only exceptional circumstances provide conditions for efficient spread of beet mosaic.

Spread of beet yellows appears to depend largely on the number of aphids which move from plant to plant within the crop, so that *A. fabae*, which moves infrequently from plant to plant, spreads little yellows. Spread of mosaic virus on the other hand depends on movement of vectors from a nearby infected source; movement from plant to plant cannot affect it because the feeding periods are usually too long. Therefore, in spreading beet mosaic, *A. fabae* is as effective as *M. persicae*. With mosaic the most important factor is probably the number of aphids from the infected source, and *A. fabae*, which often breeds in sufficient numbers to damage the seed crop, gains a numerical advantage which outweighs its inferiority as a vector.

Previously it was remarked that there seemed to be no reasonable explanation for the fact that increase of spread of mosaic virus after 1945 was not commensurate with the increase of spread of yellows virus. On the present hypothesis the increasing prevalence of the use of insecticidal treatment on the seed crop to prevent damage by *A. fabae* could have influenced the amount of spread of beet mosaic, but not yellows, because it would decrease the numbers of aphids which breed on the seed crop without preventing migrants from other sources from feeding on it, and without affecting the general level of *M. persicae* infestation in the root crops.

These considerations possibly explain why differences both within and between years in incidence of beet yellows virus can be correlated with aphid numbers and those of beet mosaic virus cannot. The influence of extraneous factors affecting the intensity of nearby sources of infection are probably so great with beet mosaic virus, that the actual number of vectors becomes of secondary importance.

This will not necessarily be true of other non-persistent viruses. Broadbent (1950) has shown that differences between years in incidence of potato *Y* virus can be significantly related to numbers of *M. persicae*. But with potatoes the sources of virus infection consist largely of plants infected in the previous year and carried over with the seed, and so are more equally distributed within and between crops, than sugar-beet viruses. Also, potatoes are a more favourable host of *M. persicae* than sugar beet; more aphids breed on the crop, and the relationship between potato *Y* virus and *M. persicae* is likely to be closer, in the absence of an alternative vector, such as *A. fabae*, which breeds prolifically on outside sources of infection.

Variation in incidence of potato leaf-roll virus more closely resembles that of beet yellows virus, because with both viruses spread is mainly by *M. persicae* and mainly within fields.

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APPENDIX

A mathematical model for the spread of virus disease by aphids

Suppose that a field contains a proportion k of infected plants randomly dispersed, and that an aphid visits t plants at random. If one of these visits is to an infected plant, the aphid is supposed to become *infective*, and plants that it subsequently visits have a probability p of becoming infected in their turn. Then the aphid will cause no new infections if it never visits an infected plant, or if after becoming infective it fails to infect any of the other plants it visits; the probability of this is

$$\begin{aligned} & (1-k)^t + kq^{t-1} + (1-k)kq^{t-2} + (1-k)^2kq^{t-3} + \dots + (1-k)^{t-1}k \\ &= (1-k)^t + k \frac{q^t - (1-k)^t}{q - (1-k)} = (1-k)^t + p_0, \quad \text{say,} \\ & \text{where} \quad q = 1-p. \end{aligned}$$

In the same way, the probability of the aphid causing just one new infection is

$$k(t-1)pq^{t-2} + (1-k)k(t-2)pq^{t-3} + \dots + (1-k)^{t-2}kp = p \frac{\partial}{\partial q} p_0;$$

and the chance of causing just two infections is

$$k \binom{t-1}{2} p^2 q^{t-3} + (1-k)k \binom{t-2}{2} p^2 q^{t-4} + \dots + (1-k)^{t-3}kp^2 = \frac{1}{2}p^2 \frac{\partial^2}{\partial q^2} p_0.$$

In general, the chance of causing n infections is

$$\frac{p^n}{n!} \frac{\partial^n}{\partial q^n} p_0 = p_n, \quad \text{say.}$$

If we write $P(x) = p_0 + p_1x + p_2x^2 + \dots$

$$\begin{aligned} \text{we have} \quad P(x) &= k \sum_{n=0}^{t-1} \frac{\partial^n}{\partial q^n} \frac{(1-k)^t - q^t}{(1-k) - q} \frac{p^n x^n}{n!} \\ &= f(q + px), \end{aligned}$$

$$\text{where} \quad f(q) = k \frac{(1-k)^t - q^t}{(1-k) - q}.$$

Then the expected number of potential infections caused by the aphid is

$$\begin{aligned} 1 \cdot p_1 + 2 \cdot p_2 + \dots &= \frac{\partial}{\partial x} P(x) \Big|_{x=1} \\ &= p[(1-k)^t + kt - 1]/k \\ &= I, \quad \text{say.} \end{aligned}$$

We shall assume that the number of potentially infective punctures caused by N aphids is simply NI .

Suppose now that N aphids visit 100 plants; by the multiple infection transformation (cf. Gregory, 1948) these will infect on the average 100 $(1 - e^{-NI})$ plants. But, again on the average, a proportion k of these will be infected to start with; therefore the increase in infection will be

$$\Delta k = (1-k)(1 - e^{-NI}).$$

The application of the formula can be made easier by the use of nomograms.

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EXPERIMENTS ON THE CONTROL OF BEET YELLOWS VIRUS IN SUGAR-BEET SEED CROPS BY INSECTICIDAL SPRAYS

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(With 2 Text-figures)

Field experiments made in eastern England between 1943 and 1951 showed that *Myzus persicae* lived on the stecklings throughout some winters, and that most plants with yellows in transplanted seed crops were from infections that occurred in the steckling bed. A larger proportion of stecklings sown at the end of July or in early August became infected than of those sown about a month later. The incidence of yellows was reduced by nicotine sprays which cleared the stecklings of aphids after autumn migrations had ceased, thus preventing spread of the disease during the winter. A greater reduction was obtained with persistent and systemic organo-phosphorous insecticides; in one experiment three applications of E605 reduced incidence to one-ninth that in unsprayed plots. However, in years when stecklings were exposed to large migrations of aphids, even plots sprayed three times had 78% of the plants with yellows. Although spraying often greatly reduces the incidence of yellows, it is unlikely to give adequate control in years and districts in which many viruliferous aphids move in the autumn. Spraying in September and October was usually more effective than in August.

The importance of seed crops of sugar beet, mangold and related plants in the epidemiology of sugar-beet yellows has frequently been stressed. Ernould (1951) records that seed crops are an important source of infection for the root crop in Belgium and Hansen (1950) in Denmark and Bjorling (1949) in Sweden have reported that root crops are often severely affected in areas where seed crops are grown. This is true also in Great Britain (Watson, Hull, Blencowe & Hamlyn, 1951), where farmers are recommended to grow seed crops only from virus-free stecklings.

In Great Britain seed crops are usually grown by setting out young plants (stecklings) raised from seed sown in a nursery bed (steckling bed) in June, July or August. They are transplanted during November to April and harvested in September. Aphids introduce and spread the virus in the steckling bed. The steckling bed is an obvious site for applying control measures, because the plants occur on approximately one-tenth of the area they occupy when set out in the second year. The incidence of yellows is affected by varying conditions that influence aphid infestation, such as sowing date, row width and density of stand. Spraying with insecticides, sowing the stecklings under cover crops to protect

them against aphids (Hansen, 1950), and separation from sources of virus and aphids are practical control measures. The experiments recorded in this paper are primarily concerned with control by spraying with insecticides.

METHODS AND MATERIALS

The experiments were made about 5 miles north of Lincoln, where sugar beet is grown regularly, but seed crops only occasionally. Each experiment extended over 2 years; in the first the sprays were applied to plots of stecklings, which were lifted and transplanted during the following February or March to another field with the same plot arrangement as in the steckling bed. The design of the experiments varied according to the treatments, but basically a factorial arrangement was used, involving all possible combinations of two or three spraying times.

The steckling beds were sown on limestone soil, after early peas (1943), bare fallow (1948, 1949), or mustard ploughed in as green manure (1944, 1945, 1946). Compound sugar-beet fertilizers were applied at the rate of $3\frac{1}{2}$ –4 cwt. per acre in 1943–7 and at 8 cwt. per acre in 1948 and 1949. Rows were 18 in. apart and plots consisted of eight rows 11 ft. long. The outer rows and 18 in. at each end were not used. Paths 18 in. wide surrounded each plot. The seed was either one breeder's line or a mixture of three lines which are constituents of variety Battles' E.

The methods of commercial practice were used for lifting and transplanting. The stecklings were pulled after loosening with a fork, laid on the ground in rows and trimmed, leaving about 2–4 in. of petiole and 3–5 in. of root. They were dibbled into the seed field along strings or marks so that the plants were correctly spaced both along and between rows. Small plants and any which were obviously damaged or had fangy roots were discarded. If the plot of stecklings contained too many plants the number required was lifted at random. The seed crop was also grown on limestone loam; 7–11½ cwt. per acre of compound fertilizer were applied either before or shortly after transplanting. Rows were 22 in. apart and plants were spaced at 18 in., and the plots contained 100–120 plants; the arrangement of paths differed from year to year. The growing points of the plants were not removed in May as is sometimes done in commercial practice. During the early summer centres of infestation of *Aphis fabae* were sprayed with nicotine.

In the steckling bed plots, the total and diseased plants were counted in 1 ft. lengths of each of four rows selected at random. The plants were examined for aphids before they were counted, so reducing the risk of aphids being disturbed and dropping to the ground. During the winter *M. persicae* was often concealed in the leaves around the growing point. The total number of aphids on the plants was recorded. In the transplanted crop all plants on each plot were examined for disease. Those infected with downy mildew were not included in the totals used for virus counts. With the transplanted seed crop in Exp. I aphids were counted on four plants in each plot; the whole plant was examined and the flower buds searched carefully.

About 100 gallons per acre of spray were applied with knapsack sprayers when the stecklings were well developed, but less was used when they were small. The upper and lower surfaces of the leaves were covered. The insecticide used at first was 0.125 % nicotine in Bordeaux mixture (1 % copper sulphate, 1-1¼ % hydrated lime, w/w). It proved effective as a contact aphicide even at winter temperatures. Later, some of the newly developed persistent insecticides were used (Schrader, 1947). Most are toxic to warm-blooded animals, but so long as operators are careful this is not a serious disadvantage, as stecklings are never fed to animals. The insecticides were used more concentrated than recommended for pest control, generally at the limit set by their phytotoxic effects.

Experiment I (1943-4)

This experiment determined the effects of sprayings in autumn and winter on stecklings sown on two dates. Six treatment comparisons were arranged factorially on sixty-four plots, but two treatments which were introduced to determine their effect on downy mildew had no effect on virus yellows and they have been omitted. The four comparisons which will be considered were:

- | | | |
|----|---|--------------------------------|
| S1 | Early sowing (5 August) compared with S2, late sowing (27 August). | |
| I | Artificial infection compared with no artificial infection. | |
| E | Early spraying with Bordeaux + 0.125 % nicotine—
22 September and 13 October | } compared with
no spraying |
| L | Late spraying with Bordeaux + 0.125 % nicotine—
22 November and 23 February | |

Artificial infections were made on 15 September by placing about twenty adult *A. fabae* fed on sugar beet infected with yellows virus, in each of five places on the appropriate plots. The stecklings were transplanted between 22 and 28 March. Aphids and yellowed plants were counted at fortnightly intervals (Tables 1 and 2 and Figs. 1 and 2).

There was a moderate infestation of *A. fabae* in the autumn, but they were all gone by the end of October. More occurred on the early than on the late sowing. An effect of the first spraying was apparent on 28 September, and the difference between the sprayed and unsprayed plots had increased by the second count on 11 October. Thereafter, the numbers of aphids decreased and the second spraying almost eliminated *A. fabae*. By 25 October only occasional aphids remained on the sprayed and unsprayed plots. The aphids on the plots for the artificial infection treatment increased the infestation; the greatest effect was seen on 11 October. A fresh infestation developed on the seed plants in the following year during May and increased considerably during June (Table 1).

The autumn infestation of *Myzus persicae* was of the same order as that of *A. fabae*. Alatae predominated on 28 September; the numbers increased during October and aphids occurred on the plants until the middle of November. Apteræ

were rare in September but increased rapidly in October and persisted throughout the winter until the plants were lifted. However, none could be found on the plants after transplanting until reinfestation occurred towards the end of May, but aphids were few throughout the summer. Spraying considerably reduced the

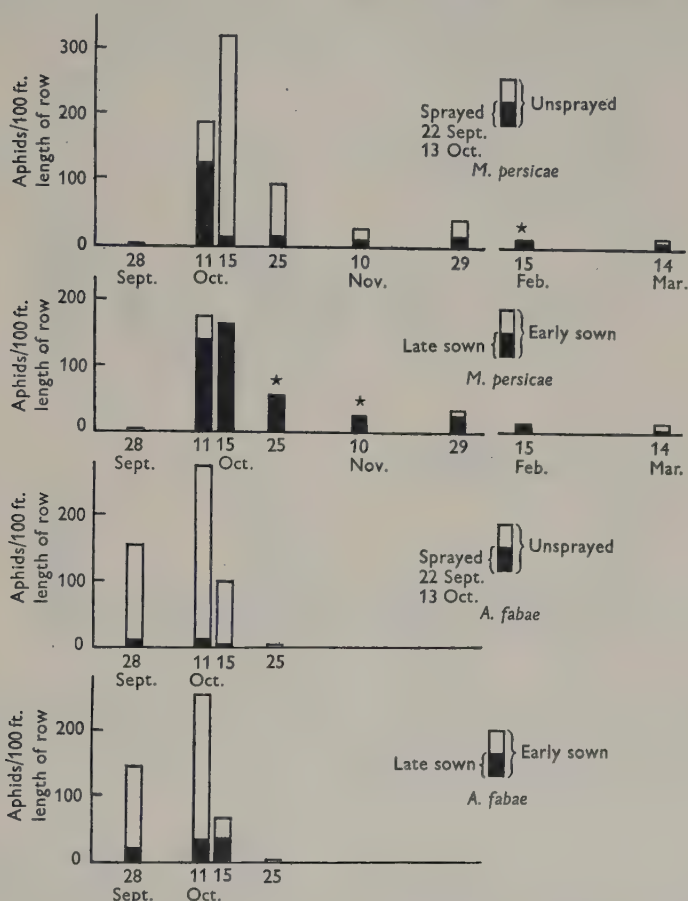


Fig. 1. Histograms showing counts of aphids at intervals during the autumn and spring 1943-4 on early-sown compared with late-sown plots and on sprayed compared with unsprayed plots. * On these occasions the count of yellows was slightly greater on the sprayed than on the unsprayed plots.

number of aphids on the stecklings; this was apparent even at the low level of infestation on 28 September (Table 1) but was more obvious on 11 October. The reduction was far greater on 15 October after the second spraying (Fig. 1). The infestation on the unsprayed plots had declined greatly by 25 October, but the beneficial effect of spraying remained obvious until February. The winter spraying

also reduced the infestation (Table 1). The infestations on the early and late sowing were similar.

The mean number of plants with yellows was 0.9% on 11 October and 4.8% on 10 November (Table 2). No counts were made in February and March because the leaves were damaged by frost. The leaves were not cut off at transplanting, when infected plants could be recognized as soon as they became established, and on

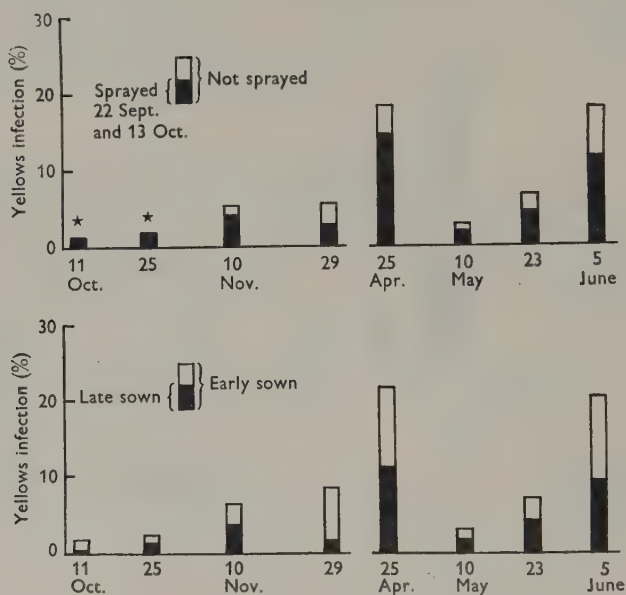


Fig. 2. Histograms showing counts of virus yellows at intervals during the autumn and spring 1943-4 on early-sown compared with late-sown plots and on sprayed compared with unsprayed plots. * On these occasions the count of yellows was slightly greater on the sprayed than on the unsprayed plots.

25 April a mean of 16.5% showed symptoms. When the old leaves died, or were covered with soil by inter-row cultivations, it was difficult to detect the disease until new leaves had developed symptoms. Hence counts during May are low, but in June they again resemble the April counts. After this there was secondary spread within and among the various plots, and treatment effects on virus counts tended to become less pronounced. Counts of infected plants at the beginning of November reasonably parallel counts in the corresponding plots the following spring.

Although they are not all significant at each recording, the effects of late sowing, early and late spraying can be seen throughout the period of the observations. Late sowing reduced yellows by about 10%. Both early and late spraying reduced it between 3 and 7% and the artificial infection treatment increased it by 2.5%.

The greater incidence on the early sown plots is associated with more *A. fabae*

on the stecklings, whereas *M. persicae* occurred in similar numbers on both sowings. This suggests that *A. fabae* may have introduced or spread the virus in the plots,

TABLE 1. *Exp. I (1943-4). Number of aphids on stecklings and seed plants*

Date	<i>A. fabae</i>				
	No. per 100 1-ft. lengths of row		Effect of treatment on total number per 100 1-ft. lengths of row		
	Alatae	Total	Late sowing	Early spraying	Artificial infestation
28 Sept.	5.9	83	-124	-146	+15
11 Oct.	5.5	143	-218	-264	+88
15 Oct.	3.0	52	-31	-100	+39
25 Oct.	0	2	-3	-3	0
9 Nov.	<1	1	-1	-2	+0.8
29 Nov.	0	0	—	—	—
Transplanted					
	No. per 100 plants				
	Alatae	Apterae			
10 May	0	0	—	—	—
23 May	4	16	—	—	—
5 June	<1	37	—	—	—
2 July	<1	1660	—	—	—
	<i>M. persicae</i>				
	No. per 100 1-ft. lengths of row		Effect of treatments on number of apterae per 100 1-ft. lengths of row		
	Alatae	Apterae	Late sowing	Early spraying	Late spraying
28 Sept.	7.0	1.2	-0.8	-2.3	—
11 Oct.	22.2	156	-34.4	-61	—
15 Oct.	19.5	168	3.9	-312	—
25 Oct.	5.9	54.3	3.9	-80.5	—
10 Nov.	1.2	20.7	7.0	-19.5	—
29 Nov.	0	28.9	-6.8	-25	-28.2
15 Feb.	0	10.9	0	1.6	-9.4
14 Mar.	0	7.0	-9.4	-6.3	-14.1
Transplanted					
	No. per 100 plants				
	Alatae	Apterae			
25 Apr.	0	0	—	—	—
10 May	0	0	—	—	—
23 May	0.8	0	—	—	—
5 June	0.2	1.8	—	—	—
2 July	0.0	2.6	—	—	—

but constitutes no proof. The difference in incidence may not have resulted solely from the aphid population developing on the plants but also from the time the

TABLE 2. *Exp. I (1943-4). Incidence of yellows in stecklings*

Date of observation	Mean percentage yellows infection	Effect of treatments on percentage yellows				
		Late sowing	Early spraying	Late spraying	Artificial infection	Standard error
11 Oct.	0.9	-1.3	0.8	—	+1.2	±0.7
25 Oct.	1.7	-1.2	0.2	—	0	±0.6
10 Nov.	4.8	-2.9	-1.3	—	+3.2	±1.4
29 Nov.	4.5	-6.9	-2.7	—	+2.5	±2.1
Transplanted						
25 Apr.	16.5	-10.5	-3.7	-6.9	+2.1	±2.0
10 May	2.5	-1.5	-0.8	-0.9	+0.8	±0.6
23 May	5.7	-3.1	-2.2	-1.9	+0.8	±0.8
5 June	14.9	-10.9	-6.5	-3.8	+2.5	±2.0

plants were exposed to infection. When the second sowing was emerging, some of the early sown plants were undoubtedly already infected, so the same *M. persicae* population could infect more plants on the early sowing. That many steckling infections can be attributed to *M. persicae* is shown by the experiments described below.

The results of an experiment made in 1944-5 are not given in detail as only 0.3 % of the plants became infected, and these were randomly distributed on sprayed and unsprayed plots.

Experiment II (1945-6)

The effect of sprays and dusts containing nicotine were compared on stecklings sown on two dates. All combinations of the following treatments were applied to a 64-plot experiment. The wet and dry application for each treatment combination were on adjacent plots and the results were analysed on a 'split-plot' basis.

- S1 Early sowing (28 July) compared with S2, late sowing (27 August).
W Wet spray of Bordeaux mixture containing 0.125 % nicotine compared with
D Dusting with a dry preparation containing copper oxychloride (16 % copper) and nicotine (4 %).
A, B, C Early, mid and late applications according to the following schedule:

	Wet spray		Dust	
	On S1	On S2	On S1	On S2
A	10, 25 Aug., 7 Sept.	15, 29 Sept.	17, 27, 31 Aug., 7 Sept.	18, 26, 29 Sept., 6 Oct.
B	15, 29 Sept.	13, 27 Oct.	18, 26, 29 Sept. 6 Oct.	13, 20, 27 Oct., 3, 10 Nov.
C	13, 27 Oct.	19 Nov., 1 Dec.	13, 20, 27 Oct., 3, 10 Nov.	19, 24 Nov., 7 Dec.

The sprays were applied at fortnightly intervals, the dustings twice as often when the weather was suitable. The dusts were applied on calm mornings when the plants were wet and were timed to give, as far as practical, a continuous cover of the foliage. The plants were transplanted in the last 2 weeks of February.

TABLE 3. *Exp. II (1945-6). Virus yellows and Myzus persicae on stecklings*

Plot treatment	In steckling bed Per 100 1-ft. lengths of row			In seed crop Per 100 plants			
	16 Oct.	17 Dec.	14 Feb.	20 Apr.	7 May	23 May	20 June
Number of <i>Myzus persicae</i>							
No spray	3.1	51.6	34.4	—	—	—	—
A	6.3	27.3	10.1	—	—	—	—
B	1.6	0.8	Nil	—	—	—	—
C	3.9	Nil	Nil	—	—	—	—
Mean	5.0	39.8	22.2	Nil	Nil	0.2†	1.4‡
Virus yellows (%)							
Mean	4.6	3.1	—	2.6	1.3	18.2	27.9

† Ten plants per plot examined.

A=30 August.

B=21 September.

‡ Two plants per plot examined.

C=15 October.

The occurrence of yellows and aphids was followed on the steckling bed and on the seed crop after transplanting (Table 3). Only 3-5% of plants showed yellows in autumn, and fewer were counted on 20 April and 7 May after transplanting. There was no peak at this time as in Exp. I, possibly because the leaves were trimmed before transplanting, but symptoms developed on the new foliage during May and June. Very few aphids had been found on the transplanted crop by this time, and these symptoms most probably result from infection contracted in the steckling bed, where aphids were fairly common during the autumn and winter.

The effects of the treatments on virus yellows are shown in Tables 4, 12 and 13. The A, B and C applications each significantly reduced the incidence of yellows, B giving the largest effect (-22.2%) and A the smallest (-7.6%). The BC interaction was significant and positive, which means that the C applications had less effect when applied to plots which had received the B applications, than when applied to those which had not. The reason for this may be seen in the aphid populations on the sprayed plots (Table 3). The counts of 17 December show that unsprayed plots had 51.6 *M. persicae* per 100 ft. of row, those receiving A sprayings only had 27.3, whereas those receiving B sprayings had 0.8. The B sprayings killed most of the aphids and there was no further reinfestation. Thus C sprayings applied after the B sprayings could show no additional effect. When applied to plots which had not had B sprayings, they killed the aphids, but only after there had been some further spread of virus.

The spray applications affected early and late sowings similarly, but the early sowing had more infected plants than the late. The wet and dry applications of

insecticide were equally effective. The cumulative effects of the A, B and C applications are shown in Table 4.

TABLE 4. *Exp. II (1945-6). Incidence of yellows on sprayed plots*

Spray treatment	Infection with virus yellows (%) 20 June	
	Stecklings sown, 28 July	Stecklings sown, 27 Aug.
None	68.5	37.2
A	55.9	40.5
A+B	22.4	10.2
A+B+C	15.4	7.0

± 5.97

Experiment III (1946-7)

In this experiment the effect of different times of application of sprays containing nicotine, DDT and the two insecticides together were investigated. The treatments were:

E	Early sowing, 23 July.
L	Late sowing, 23 August.
N	Bordeaux mixture + 0.125 % nicotine.
D	Bordeaux mixture + 0.1 % DDT compound (Guesarol 33 Wettable Powder containing 33 % DDT).
ND	Bordeaux mixture + both insecticides.
(-), A, B, C	No spraying, early, mid and late spray applications respectively according to the following schedule:

	On early sowing	On late sowing
A	15, 29 Aug.	6, 23 Sept.
B	6, 23 Sept.	11, 28 Oct.
C	12, 28 Oct.	11 Nov.

The plot treatments were the eight combinations of the three times of spray application for nicotine, DDT and both nicotine and DDT together, making twenty-four spray treatments which were repeated on the early- and late-sown plots. The forty-eight plots were arranged in four blocks of twelve plots; the sowing date effect and also the ABC and SABC interactions were confounded with block differences.

The plants were transplanted in the third week of April. Infected plants were counted on 21 June, when the mean infection was 26.3 %; and on 3 July, when it was 35 %. The data of the later count were analysed and the results are given in Tables 5, 12 and 13. Nicotine spray reduced the percentage only slightly, but in conjunction with DDT reduced it more. DDT alone increased the incidence by 15.7 % on the early sowing. Whether this is because DDT increased the activities of aphids before killing them, or whether it increases the aphid population by killing predators cannot be concluded from the observations.

The sprays had significant effects only on the early sowing. Each spray application reduced incidence (Table 13), but only the late (C) application gave a significant reduction on average.

TABLE 5. *Exp. III (1946-7). Effects of spray treatments on incidence of virus yellows*

	Percentage virus yellows on 3 July			Mean response to spraying		
	Early sown	Late sown	Mean	Early sown	Late sown	Mean
No spraying	58.5	11.2	34.9	—	—	—
Nicotine	48.5	13.1	30.8	-10.0	1.9	-4.1
DDT	74.2	15.8	45.0	15.7*	4.6	10.1*
Nicotine + DDT	43.7	12.5	28.1	-14.8*	1.3	-6.8
Standard errors	—	—	—	± 5.9		± 4.2
Mean	55.9	13.4	34.7	—	—	—

* Significant at 5 % level.

Experiment IV (1948-9)

The effects of five different insecticides and three times of application were compared. The seed was sown on 26 July and the sprays were applied:

- A Early, 30 August.
- B Middle, 21 September.
- C Late, 15 October.

The following materials were applied at about 100 gallons per acre:

Pestox III (bis(bisdimethylamino) phosphonous anhydride). This proprietary product contained 47% of the anhydride and was used as a 0.5% solution in water plus sulphonated lorol (2½ lb. of the anhydride per 100 gallons). This is about half the concentration now recommended as an aphicide, whereas all other materials were used in excess of the normal rates of application.

E 605 (Parathion, 70% diethyl *p*-nitro phenyl thiophosphate). The spray contained 0.125% *E 605* and 0.5% white miscible oil in water with sulphonated lorol. The *E 605* tended to settle out in globules and the spray had to be stirred continuously.

HETP (hexaethyltetraphosphate). 0.125% in water with sulphonated lorol.

'*Octaklor*' (Chlordane, Velsicol 1068). 0.125% in water with sulphonated lorol and 0.5% white miscible oil.

Nicotine (alkaloid). 0.125% in water with sulphonated lorol and 0.5% white miscible oil.

Each treatment combination of the five sprays and the eight application times was repeated on two plots and the eighty plots were arranged in four blocks, the ABC interaction being confounded with block differences.

On 10 February there were more *M. persicae* than in the previous experiments.

As the stand of plants was rather irregular, counts of aphids per foot length of row would have been erratic, so the infestation was estimated by determining the proportion of infested plants in one row of each plot (Table 6). The plants were transplanted between 16 and 23 March. They were dipped in nicotine solution after lifting to avoid transferring aphids to the seed-crop plots. No *M. persicae* was observed on the seed plants on 23 May.

TABLE 6. *Exp. IV (1948-9). Percentage of plants infested with Myzus persicae on 10 February 1949*

Time of spraying	Spray material					Mean	Mean treatment effect
	Pestox III	E605	HETP	Octaklor	Nicotine		
	Percentage of plants infested with <i>M. persicae</i>						
None	—	—	—	—	—	31.7	—
A	18.1	43.7	49.8	73.8	65.0	49.8	0.5
B	36.2	8.4	23.8	45.7	25.6	26.8	-9.2**
C	20.0	0.5	21.0	43.0	20.3	18.1	-24.4**
AB	18.9	10.2	53.2	22.9	58.0	31.2	-2.3
AC	10.4	0.0	4.6	59.3	2.7	9.8	-9.1**
BC	5.9	0.0	18.7	33.0	8.7	10.0	0.7
ABC	3.5	0.0	0.9	35.5	4.4	5.1	—
Mean	14.8	4.1	21.1	44.6	22.7	21.1	—

Differences from no spraying (%)

-16.9** -27.6*** -10.6 +12.9 -9.0 — —

** Significant at 1 % level.

*** Significant at 0.1 % level.

A=30 August.

B=21 September.

C=15 October.

Plants with yellows were counted on 23 May and 8 June when the mean infections were 53.7 and 68.3 % respectively. The results for the latter count were transformed to mean angles for statistical analysis. In Table 7 they have been converted back to percentages and significant effects are indicated.

The sprays clearly affected the numbers of plants infested with *M. persicae* in February (Table 6). E605 was the most effective, for when a C spraying had been used in conjunction with any other, the plot was free from aphids. Pestox III was also effective and there is a strong indication that the later spraying with HETP and nicotine had an effect, but Octaklor increased infestation. The result with nicotine is similar to that obtained in previous experiments. The late C application was the most effective, the effect of the B application is significant, but with all sprays the A application had little effect.

The aphid infestation built up from the winged aphids that reached the plants in the autumn. The C spray application was effective because its effect lasted until the autumn migration ended. The effect of the B spraying with E605 lasted long enough to reduce the final infestation, but the effect of the A application did not. The AC interaction effect is significant, which shows that the effect of the two applications together is greater than the sum of the effects of each used separately.

The incidence of yellows in the second year was reduced significantly by Pestox III, E605 and HETP (Table 7). It was slightly less on the plots sprayed with nicotine than on the unsprayed controls. It was slightly higher on those sprayed with Octaklor, a result similar to that obtained with DDT in Exp. III. The effects of the sprays on yellows parallel those on aphid infestation (Table 6). The effects of the B and C spraying are significant, that of the C being the greater (Table 13). The interaction effect of the A and C sprayings is not, however, significant.

TABLE 7. *Exp. IV (1948-9). Incidence of yellows infection on 8 June 1949*

Time of spraying	Spray material					Mean
	Pestox III	E605	HETP	Octaklor	Nicotine	
	Percentage virus yellows					
None	—	—	—	—	—	85.0
A	81.3	69.7	89.0	94.2	95.5	87.2
B	46.2	42.2	80.2	95.8	88.0	73.5
C	78.3	39.7	65.2	75.6	63.7	64.8
AB	59.8	47.2	75.6	90.9	98.7	78.1
AC	64.8	22.0	43.7	94.3	54.5	57.7
BC	28.1	14.5	52.2	93.5	64.5	51.4
ABC	23.2	9.6	41.3	62.5	79.4	42.0
Mean	54.8	33.5	65.2	88.4	80.8	68.3
	Differences from no spraying (%)					
	-30.2***	-51.5***	-19.8**	3.4	-4.2	—
** Significant at 1% level.			*** Significant at 0.1% level.			
A=30 August.		B=21 September.		C=15 October.		

All plants were harvested, and after thrashing the seed was cleaned in a winnower and over an inclined, moving belt before weighing. The yields of cleaned seed are given in Table 8. The mean yield, 17.2 cwt. per acre, is rather low because the crop suffered severely from drought. The E605 sprayings gave a mean increase in yield of 4.9 cwt. per acre over the unsprayed plots. Treatment yields above 19.6 cwt. per acre are significantly greater than the unsprayed plot yield; thus a beneficial effect was obtained with the BC and ABC sprayings of Pestox III, with all the spray treatments with E605 except A and with the ABC spraying with HETP. No significant effect was obtained with the nicotine sprayings and Octaklor depressed yield.

Assuming the treatments had no effect on yield other than through their effect on yellows, which may be partly due to delayed infection as well as a reduction in the actual percentage of plants infected, the regression coefficient of seed yield on yellows incidence on 8 June is $b = -0.0878 \pm 0.0097$ cwt. per acre for 1% virus yellows infection. Thus with a mean yield of 17.24 cwt. per acre an increase of 10% plants with yellows decreased yield by 0.88 cwt. per acre.

TABLE 8. *Exp. IV (1948-9). Yield of seed from plots receiving different spray treatments*

Time of spraying	Spray material					Mean
	Pestox III	E605	HETP	Octaklor	Nicotine	
	Yield of seed in cwt. per acre					
	(± 1.66)					
None	—	—	—	—	—	15.9
A	16.6	18.7	14.0	14.9	11.8	15.1
B	16.5	21.0	17.9	14.1	16.6	17.1
C	14.9	20.6	17.9	15.1	18.2	17.2
AB	17.1	20.6	17.0	14.5	15.4	17.0
AC	16.9	20.4	17.4	17.1	19.2	18.3
BC	20.3	22.5	15.1	14.9	15.3	17.7
ABC	21.6	21.7	23.3	14.3	17.6	19.6
Mean	17.7	20.8	17.5	15.0	16.3	17.2
	(± 0.63)					
Increase over no spray	1.8	4.9**	1.6	-0.9	0.4	—
	(± 0.97)					

** Significant at 1 % level.

A = 30 August.

B = 21 September.

C = 15 October.

Experiment V (1949-50)

Experiments in the glasshouse showed that sprays containing 0.05 % E605 emulsified with Triton N, caused considerable leaf injury, but 0.1 % E605 could be used without excessive injury when applied in a 1 % mixture of miscible white oil in water, with or without 0.4 % Lissapol N. Globules come to the surface of this mixture, which needs constant agitation.

The persistence of toxicity with E605 was measured by placing *M. persicae* in dishes on leaves cut from sprayed plants at intervals after spraying. Aphids were not killed when placed on leaves sprayed with well-emulsified solutions 8 days previously, but they were killed when placed on leaves sprayed 15 days previously with 0.1 % E605 in oil suspension or formulated with Lissapol N.

A field experiment was arranged to test various formulations of E605 at 0.09 %, in addition to Pestox III at 0.5 and 1.0 % and Pestox XIV (Hanane, bis(dimethyl-amino)-fluorophosphine oxide) at 0.2 %. The eight treatments (Table 9) were replicated in six randomized blocks. Sprays were applied on 7 September, 12 October and 8 November, corresponding to the ABC treatment of Exp. IV. The seed was drilled on 29 July, and patches were resown on 22 August because of losses from wireworms. The seedlings were transplanted on 27-29 March and infected plants were counted on 21-22 June. The percentages were analysed after angular transformation and the results are given in Table 9.

All treatments except Pestox III at 0.5 % significantly reduced yellows, but 80 % infected plants occurred in the best plots. Pestox XIV and the two best E605 sprays were significantly better than Pestox III at 0.5 %, but there are no significant differences between the other treatments.

TABLE 9. *Exp. V (1949-50). Incidence of yellows in sprayed stecklings*

Treatment	Virus yellows (%)	Decrease from control (unsprayed) (%)
Pestox III, 0.5 % + Lorol	91.7	- 4.0
Pestox III, 1.0 % + Lorol	86.9	- 8.8
E605 (old material), 0.09 % + misc. oil	87.9	- 7.8
E605 (old material), 0.09 % + Lissapol N	88.9	- 6.8
E605 (old material), 0.09 % + misc. oil + Lissapol N	82.4	- 13.3
E605 (new material), 0.09 % + misc. oil	81.4	- 14.3
Pestox 14, 0.2 %	78.3	- 17.4
Unsprayed control	95.7	—

The results of the 1950-1 experiment are not given in detail. Very few aphids were present on the nearby beet crops in the autumn of 1950 and the mean infection in the stecklings was only 1.4%.

Exp. VI (1949-50). Spraying stecklings at various centres

Spraying experiments were arranged on six commercial steckling beds in the eastern counties in the autumn of 1949. The treatments were the eight factorial combinations of no spray, early (A), mid (B), and late (C) applications. Two insecticides were used; Pestox III at 0.5 % of the active ingredient, in water and sulphonated lorol; and E605 at 0.09 % active ingredient in water with 1 % white miscible oil + 0.2 % Lissapol N.

The dates when sprays were applied at the various sites were:

Site	A	B	C	Plant size at time of first application
(1) Willoughton (Lindsey)	29 Aug.	29 Sept.	31 Oct.	2 leaves
(2) East Heckington (Kesteven)	5 Sept.	3 Oct.	1 Nov.	4-8 leaves
(3) Fleet (Holland)	5 Sept.	3 Oct.	1 Nov.	4-8 leaves
(4) Tydd St Giles (Holland)	5 Sept.	3 Oct.	1 Nov.	2 leaves
(5) Wrestlingworth (Cambs)	16 Sept.	13 Oct.	4 Nov.	2-4 leaves
(6) Fenstanton (Hunts)	16-20 Sept.	13 Oct.	4 Nov.	2-4 leaves

Very few, or no, aphids occurred at any site except Willoughton, where there was a moderate infestation of both black and green aphids between the first and second sprayings.

Samples of about 200 plants were lifted from each plot in February or March and taken to Dunholme, where they were planted in the same plot arrangement as at the original site. Thus, in the transplanted experiment, site effects were confounded with blocks, each block containing the sixteen factorial treatments completely randomized. The plots contained 144 plants. Surviving plants and those with yellows were counted in the middle of June (Table 10). The figures for percentage disease have been subjected to angular transformation for statistical analysis, but for presentation have been reconverted to percentages.

Mean infection was 16–25% at Fleet, Fenstanton and Wrestlingworth, and 49–78% at Willoughton, Tydd and East Heckington. The early (A) spray application considerably reduced infection at Willoughton and at East Heckington but gave less, or no, response at the more southerly centres, although it approached significance at Tydd. The mean response to A over all centres is not significant at the 5% level, as the standard error is higher than the others because of the large sites \times A interaction.

The B application produced smaller, but significant, reductions in incidence at Willoughton, Tydd and Fleet, and the effect at Wrestlingworth approached significance at the 5% level. The mean reduction of 12.9% over all centres was significant.

TABLE 10. *Exp. VI (1949–50). Effect of spray application in the autumn of 1949 on the incidence of virus yellows at six centres in eastern England*

Spray treatment	Fleet	Fen- stanton	Wrestling- worth	Willough- ton	Tydd	E. Heckington	Mean
Percentage virus yellows							
—	23.2	19.4	14.8	79.3	83.0	65.7	47.0
A	25.4	13.7	15.0	32.7	73.2	32.4	31.2
Response	2.2	-5.7	0.2	-46.6	-9.8	-33.3	-15.8
—	30.8	19.1	19.5	65.5	86.3	52.5	45.5
B	18.4	13.9	10.7	47.8	68.8	45.5	32.6
Response	-12.4	-5.2	-8.8	-17.7	-17.5	-7.0	-12.9
—	21.6	15.8	16.8	55.4	78.3	51.0	38.8
C	27.2	17.0	13.1	58.2	78.1	46.8	39.2
Response	5.6	1.2	-3.7	2.8	-0.2	-4.2	0.4
E605	22.9	15.0	16.1	66.0	75.4	49.0	39.7
Pestox III	25.7	17.7	13.6	47.2	80.9	49.0	38.2
Difference	2.8	2.7	-2.5	-18.8	5.5	0	-1.5
Mean	24.4	16.4	14.9	56.8	78.3	49.0	39.0

The C application produced no significant effect at any centre. This is different from results of previous years, when a good response was obtained from late spraying, and is explained by the earlier end of aphid migration in 1949.

At five centres E605 and Pestox III behaved similarly, but at Willoughton, especially with the first application, Pestox III was superior. The circumstances probably favoured obtaining the full benefit of the systemic effect of Pestox III. The plants were small but growing rapidly when the spray was applied. When aphids arrived a few days later, there was new growth which was not covered with E605 but which was toxic with Pestox III because of its systemic effect.

At Fenstanton and Wrestlingworth aphids were counted at fortnightly intervals on four 1 ft. lengths of row on each plot. On 31 October at Wrestlingworth one alate and one apterous *M. persicae* were found, and these were the only ones seen. In spite of this minute infestation and spraying, a mean of about 23% infected

plants occurred at both sites. Infection was probably caused by alate aphids feeding on the plants and then leaving without colonizing the bed. It might be suspected that the plants became infected after transplanting at Dunholme, but counts at the same time on other plots in the same field showed no spread of yellows.

The mean cumulative effect of the three spray applications are shown in Table 11. Averaged over all centres the A application alone reduced infection by 21% and the B application alone by 19%; together they reduced it from 58 to 24%. No further advantage was obtained this year from the third application.

TABLE 11. *Exp. VI. Cumulative effect of three spray applications on percentage yellows*

Treatment	Mean virus yellows infection (%)
O	57.7
A	36.2
B	39.0
C	51.0
AB	23.7
AC	37.2
BC	41.0
ABC	27.5

DISCUSSION

Field counts of plants infected with virus diseases are seldom unequivocal, and this is particularly true of beet yellows in winter and spring. The most pronounced and distinctive symptoms develop on seed crops at the end of May or during June. This is evidently the best time to measure the degree of control obtained by spray applications in autumn. The diseased plants seen in the crop at this time are the result of infections occurring in the autumn and winter while the spray treatments are still having an insecticidal effect. The observations of aphids in Exps. I and II also support this, for very few were found on the transplanted seed crop before infected plants were counted in June, and it is doubtful whether there would have been time for infections caused by these aphids, or the alatae of which they were the progeny, to have produced symptoms. In commercial fields throughout Lincolnshire observed in the autumn of 1943 and spring of 1944, aphids were found in steckling beds up to the end of November but none was found on the seed crops until the first week of June, by which time the diseased plants had increased to 60% from a mean of 3% in the steckling beds in November. Much other evidence has come from experiments with stecklings grown in isolation to show that, near Lincoln, little or no spread occurs in transplanted seed crops in the spring.

Fewer late-sown stecklings contracted infection than early-sown in each of the experiments (Table 12). This is the consequence of early-sown plants being exposed to winged aphids for a longer period. Had the late-sown seed germinated after the peak of aphid movement, the effect might have been more pronounced. On the

other hand, if seedlings from a late sowing emerge when alate aphids are plentiful, the plants become more heavily infested than an earlier sowing in which the plants have filled the rows. This has been known to occur in Denmark (Hansen, 1950), but will evidently not be a usual occurrence in this country.

The spread of virus in a steckling bed results not merely from the number of aphids present, but from the number of viruliferous aphids moving and feeding on the crops, which in turn depends on the aphid population, the number of virus sources and the opportunity for the aphids to pick up the virus from them. An insecticide might reduce the incidence of a virus disease by repelling viruliferous winged aphids, discouraging them from feeding on the crop and thus from causing infections. It would also reduce the number of aphids becoming infective by

TABLE 12. *Effect of sowing date on percentage virus yellows.*
Summary of all experiments

	Sowing dates	Effect of sowing date (late-early) on virus yellows (%)	Mean virus yellows (%)
Exp. I (1943-4)	5 Aug., 27 Aug.	-10.9** \pm 2.0	14.9
Exp. I (1944-5)	3 Aug., † 1 Sept.	—	0.3
Exp. II (1945-6)	28 July, 27 Aug.	-13.4** \pm 2.99	27.9
Exp. III (1946-7)	23 July, 23 Aug.	-42.5** \pm 4.92	34.7
Exp. IV (1948-9)	26 July	—	68.3
Exp. V (1949-50)	29 July	—	86.6
Exp. VI (1949-50)	Various	—	39.0
Exp. VI (1950-1)	17 July	—	1.4

** Significant at 1 % level.

† Dry until 19 August.

feeding on infected plants within the crop. There is, however, a danger that materials which are not powerful repellents or are not rapidly lethal might, by disturbing the feeding habits of the aphids, increase the spread of virus as appears to have happened with DDT (Exp. III) and Octaklor (Exp. IV). Thus, to be effective, a repellent will have to prevent aphids from feeding on the plants and not merely make them feed for shorter periods on more plants. In these experiments there is no evidence that any of the insecticides have exercised a repellent effect on aphids entering the crop from outside sources.

Secondly, an insecticide might kill the aphids before they can feed long enough to infect a plant, or more than one plant. It will also reduce the number of aphids, both of those that enter and those that are born in the crop, that become infected by feeding on diseased plants within the crop. The experiments described have shown that many plants to which insecticides have been applied at short intervals from emergence until aphid flights ceased in November have in some years contracted infection, but sometimes spraying has greatly reduced the incidence of yellows. In the earlier experiments four or six applications of nicotine spray reduced infections by a factor of up to five times, although in Exp. IV three applications had little

effect. The organic-phosphorus sprays gave better results; three applications of E605 in 1948-9 reduced infection by a factor of nine times, a result of practical significance. Infections are likely to be less reduced in experiments in which small sprayed plots are intermingled with unsprayed plots, than when large crops are sprayed all over.

In some experiments early applications were more effective than late ones, whereas others reversed this. The difference presumably depends on when the migrant aphids arrive. Thus, there was a marked response to the early spraying at Willoughton and East Heckington (Exp. VI) and to the middle spraying of Exp. II, while in others (Exps. III and IV) the observations on aphids indicate that the sprays have reduced yellows because they reduced spread within the steckling bed by lowering the population of apterous aphids, particularly in the latter part of the autumn and early winter. The organo-phosphorus compounds could do this not

TABLE 13. *Effects of spray treatments on percentage virus yellows.*
Summary of all experiments

	Exp. I (1943-4)	Exp. II (1945-6)	Exp. III (1946-7)	Exp. IV (1948-9)	Exp. VI (1949-50)
	Effect on virus yellows (%)				
A	-6.5	-7.6*	-3.0	-1.9	-15.8
B	No treatment	-22.2**	-3.8	-12.9**	-12.9**
C	-3.8	-13.7**	-5.9*	-27.1**	0.4
Interactions:					
AB	—	1.4	-2.5	0.0	1.1
AC	—	-2.4	-3.5	-6.0	2.5
BC	—	9.0**	-3.9	-0.7	3.2
Standard error	± 2.0	± 2.99	± 2.46	—†	—†
Mean infection (%)	14.9	27.9	34.7	68.3	39.0

* Significant at 5 % level.

** Significant at 1 % level.

† Calculated on transformed data; significance of percentage effects is indicated.

only because of their insecticidal efficiency, but because their persistence would make them more effective than nicotine against reinfestation for some time after the spray application. As the spread of aphids and virus within the crop can be prevented or greatly reduced by timely applications of organo-phosphorus insecticides, the level of infection on sprayed plots will depend, in the absence of a rapidly lethal effect on aphids, on the numbers of viruliferous winged aphids that enter them. With many migrants and many virus sources, the final level of infection in the sprayed stecklings may be high, as in Exp. V. A more effective control might be obtained by spraying the sources of viruliferous aphids, but this is impractical because the source of viruliferous aphids at the time stecklings are being raised is the extensive acreage of sugar beet, mangold and fodder beet, root and seed crops.

Thus, although spraying with existing insecticides may be developed into a valuable control measure, and in conjunction with other control measures, such as siting away from virus sources and raising stecklings under cover crops, may give comparatively healthy crops, it is unlikely to achieve a complete control on open beds in years and districts in which many viruliferous aphids move in the autumn.

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TESTS OF SYNERGISM BETWEEN NICOTINE AND THE PYRETHRINS

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Synergism between nicotine and pyrethrum applied by injection to adult *Oncopeltus fasciatus* Dal. has been reported by Turner (1951).

When these insecticides were applied alone and as a mixture to adult *Tribolium castaneum* Hbst., using a dipping technique, the data indicated that independent joint action occurred. Similar action could be eliminated because the two insecticides had varying relative potency.

Since the effect of the pyrethrins has been short-lived in some insects, it was postulated that the absence of synergism might be caused by failure of the nicotine to reach a site of action while the pyrethrins were still acting.

Application of nicotine, followed later by treatment with pyrethrins, gave evidence of synergism. A test in which the interval between treatments was varied from $\frac{3}{4}$ to 6 hr. showed that toxicity was greatest with the shortest interval between applications, and evidence of synergism had practically disappeared after 6 hr. The maximum amount of synergism observed was about twofold.

Evidence for synergism between nicotine and the pyrethrins has been reported by Turner (1951). In those tests the two insecticides were applied separately and jointly to adult milkweed bugs (*Oncopeltus fasciatus* Dal.) by injection. The degree of synergism observed ranged from none in one mixture to the equivalent of twice the dosage, when calculated by the method of Wadley (1945). When dosages of nicotine causing low mortality were followed by amounts of pyrethrins of low toxicity 1 day later, a high mortality also resulted. When the pyrethrins were injected first and nicotine applied a day later, there was no evidence of synergism. Apparently the nicotine acted in some way to make the insects more susceptible to the pyrethrins. The possible magnitude of this synergism was large enough to justify additional study. It was also necessary to extend the work to external application, since both Menusan (1948) and Beard (1949) have shown that the toxicity of insecticides by injection is not necessarily related to its effectiveness by external application.

There is apparently some difference of opinion as to the definition and usage of the term 'synergism'. Macht (1929) applied the term to describe pharmacological effects of mixtures of drugs which were not a simple summation of the effects produced by individual compounds. He mentioned both 'potentiation' and

* The experimental work was done by Neely Turner while on exchange at the Department of Insecticides and Fungicides, Rothamsted Experimental Station.

'antagonism', but all of his examples were of the 'potentiation' type. Bliss (1939) restricted synergism to increased toxicity, stating that it is 'characterized by a toxicity greater than that predicted from experiments with the isolated constituents'. Finney (1952), Horsfall (1945), and Wadley (1945) accepted the Bliss definition.

Inman (1929) introduced the term 'activator' to describe a material which increased the physical efficiency of an insecticide. Haller, McGovran, Goodhue & Sullivan (1942) used 'synergist' and 'activator' synonymously to describe the action of sesamin in increasing the toxicity of pyrethrins. Cox (1943) restricted the meaning of synergism, including only materials which were toxic when used alone. He used the term 'activator' for a substance which had little or no toxicity of itself, but increased the effectiveness of an insecticide. Busvine (1951) has used the term 'adjuvant' to define the slightly toxic materials which increase the toxicity of the pyrethrins. Shepard (1951) has reviewed the literature and discussed the subject briefly. He suggested that the term 'activator' be used, and defined it as 'a substance which renders another more active'. His reason for the choice of terms was 'because the particular type of joint action in most cases is not known'. However, he suggested no criterion for 'more active', and his change in terminology adds no light to the problem.

In this paper the term 'synergism' is used as defined by Bliss (1939), that is, the mixture of synergistic materials has a toxicity greater than that predicted from experiments with the isolated constituents, each of which has an appreciable toxicity when used alone.

METHODS

The method selected for external application was the dipping technique of McIntosh (1947). The test insects were adults of *Tribolium castaneum* Hbst. Twenty insects were used in each dipping tube, and those adhering to the rubber stopper were discarded. Each lot of insects was immersed in the dipping fluid for 1 min. plus an average of 20 sec. required to drain the tube. The bath was held at 27° C., and the insects were reared and stored after dipping at the same temperature. Mortality was determined 48 hr. after dipping.

The nicotine was the 95% alkaloid, applied in a water solution with 10% (v/v) acetone and 0.1% (w/v) sulphonated lorol. The acetone and sulphonated lorol did not affect the toxicity of the nicotine, but were added because they were needed in the pyrethrins formulation. A 25% concentrate of the pyrethrins was dissolved in acetone and added to water containing the sulphonated lorol.

The present definition of synergism suggests the only method of determining whether or not it has occurred. The mortality from joint application must exceed significantly that expected for independent or similar joint action.

Types of joint action. Although three types of joint action have been recognized, there is still no general agreement as to the exact boundaries between them or how

precisely each should be defined. They are independent action, similar action and synergistic (or antagonistic) action. Each ingredient is assumed to be active individually, with a toxicity which is defined quantitatively in terms of the dosage-mortality curve for each poison applied separately. In some cases, a non-toxic constituent may increase markedly the toxicity of a poison. Whether this relation should be considered as synergism does not concern us here.

With independent joint action, neither poison is supposed to increase or diminish the toxicity of the other. However, individuals that are more susceptible to one poison may also be more susceptible to the other. The extent to which the minimum effective doses are correlated limits the kill expected from a combined dose of the two constituents and has modified the definition of independent joint action. Bliss (1939) would permit the correlation to extend from 0 to 1. In the latter case, the toxicity of the mixture would be the same as that of the more toxic ingredient. Plackett & Hewlett (1948) extend the concept of independent joint action to include negative correlation in the susceptibility to two poisons, so that an individual that is more susceptible than the average to one poison will tend to be less susceptible than the average to the other. In their view, the correlation in susceptibilities may vary from $r = -1$ to $r = +1$. If $r = -1$, the toxicity of the combination would equal the sum of the proportions killed by each poison administered separately, or 1, whichever is the smaller.

The second type of joint action has been termed similar. The two poisons are assumed to act upon the same chemical and physiological systems within the animal so that a fixed quantity of one poison can replace one unit of the other poison. To validate the hypothesis of similar action, toxicity data should meet two requirements. The first is that for a given insect population of one species the variation in susceptibility to the two poisons must be equal. This means that their respective dosage-mortality curves, of probit kill plotted against the log effective dose, must give parallel straight lines within the sampling error.* The second requirement is that the *relative* toxicity of the two poisons must remain the same in a given insect population despite variations in its susceptibility to toxicants. If a change in the susceptibility to one poison were not paralleled by a similar change in the susceptibility to the other poison, as reflected by the LD 50 for example, it would be evident that a given quantity of one poison could not be substituted directly for a fixed amount of the other. Hence, stability in the relative potencies of two poisons within a given stock or species is essential to the hypothesis of similar joint action.

* Since this manuscript was prepared, Hewlett & Plackett have published a short paper on similar action (*Nature, Lond.*, **169**, 198-200, 1952). They propose that similar action may occur when the probit-log-dose lines for the two compounds applied individually are not parallel. They re-define similar action, requiring that the response is produced by causing the same system to react or fail, and that joint application shows that neither influences the behaviour of the other. This is a sufficient change in the concept of similar action to require more discussion and treatment than the data on nicotine and the pyrethrins justify.

Moreover, nicotine has already been shown (Turner, 1951) to influence the behaviour of the pyrethrins, so that according to the new definition the two could not act similarly.

Under some conditions, higher mortalities would be expected from similar joint action than from independent joint action, but this is not necessarily true, even if negative correlations in susceptibility are excluded (Plackett & Hewlett, 1948). A greater observed mortality than that expected on the hypothesis of similar action is not a certain demonstration of synergism.

Mortality in excess of that expected by either independent or similar joint action, whichever is the greater, may be called synergistic. The present paper is concerned only with testing its presence, not in developing or applying equations for predicting the toxicity of a mixture from that of its ingredients. The present data are not suitable for evaluating the several quantitative formulations of synergistic action now in the literature.

RELATIVE TOXICITY OF PYRETHRINS AND OF NICOTINE

Under our test conditions, preliminary trials showed nicotine to be about 0.01 times as toxic as the pyrethrins. This ratio of the two poisons has been assumed in designing the experiments reported here. Original data on the toxicity of the two poisons when applied separately are shown in Table 1. These cover tests on seven different days and in no case was there any natural or control mortality attributable to the carrier in which the pyrethrins or nicotine were applied.

It is evident without calculation that the susceptibility of the beetles varied markedly over the seven test days. On several days, the resistance of the insects increased so markedly that a disproportionate number of the doses fell in the lower end of the toxicity scale where mortality was erratic. Other experiments with these poisons have shown a greater kill at very low doses than would be expected from dosage-mortality curves which fitted the remaining data quite satisfactorily. A similar condition seems to have occurred here, especially when the data were plotted in terms of log-concentration and probit kill. For this reason, the starred values in Table 1 have been omitted in computing the dosage-mortality curves or relative toxicities in Table 2.

Separate dosage-mortality curves were computed by the method of maximum likelihood for each poison (Bliss, 1951; Finney, 1952). The slopes of the curves for nicotine and for the pyrethrins were compared within each day or group of successive tests. In those on 7 and 8 February, the four curves were satisfactorily parallel and have been represented by a single regression coefficient. The tests on 3 and 5 April were handled separately, in part because of the high variability about the fitted straight lines in the data on 3 April. In the last three tests resistance to the poisons decreased so much that, of the six potential curves, only three provided useful estimates of the slope, all agreeing within the sampling error. In the absence of a better estimate these slopes have been used in computing the relative toxicities on 10-16 April.

Homogeneity within an assay has been measured by the assay χ^2 , which includes

TABLE 1. Original data for each replicate on the separate dosage-mortality curves of nicotine and of pyrethrins for *Tribolium castaneum* by dipping, showing the number of individuals exposed in each lot (N) and the percentage kill (%)

Poison	Concentration, %	Response observed on													
		7 Feb.		8 Feb.		3 Apr.		5 Apr.		10 Apr.		13 Apr.		16 Apr.	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%
Nicotine	1.0	15	73.3	15	66.7	15	93.3	14	92.9	15	26.7	18	33.3	17	11.8
		15	66.7	13	46.2	17	82.4	17	76.5	15	13.3	15	6.7	16	18.8
	0.5	14	42.9	12	25.0	15	40.0	9	88.9	17	23.5	18	0*	15	0*
		15	40.0	13	15.4	14	21.4	18	66.7	18	5.6	12	0*	19	0*
	0.25	16	6.2	14	14.3	17	47.1	15	46.7	17	0	18	0*	16	6.2*
		15	13.3	14	14.3	16	12.5	16	43.8	19	5.3	16	12.5*	17	0*
Pyrethrins	0.125	13	0*	15	6.7*	15	20.0	16	12.5	16	6.2	19	5.3*	16	0*
		14	14.3*	14	14.3*	16	25.0	16	0	13	0	19	5.3*	18	0*
	0.01	12	75.0	13	84.6	13	0	15	53.3	19	84.2	19	47.4	19	15.8
		15	66.7	15	66.7	13	30.8	16	68.8	18	38.9	19	10.5	17	5.9
	0.005	11	54.5	15	26.7	16	0	13	69.2	19	68.4	16	25.0	18	0*
		14	28.6	15	26.7	15	26.7	15	40.0	17	41.2	16	37.5	17	0*
	0.0025	14	7.1	15	0	16	12.5	11	54.5	16	31.2	17	11.8	19	0*
		15	6.7	15	0	13	7.7	17	11.8	16	31.2	17	11.8	17	5.9*
	0.00125	15	0*	15	20.0*	15	0	15	6.7	18	5.6	15	6.7	17	5.9*
		15	6.7*	15	6.7*	16	0	19	15.8	17	11.8	19	0	18	0*

* Omitted in computing dosage-mortality curves or toxicities shown in Table 2.

TABLE 2. Toxicity of nicotine relative to that of pyrethrins and median lethal dose for pyrethrins, with the statistics computed from the data in Table 1 ($n = \text{degrees of freedom}$)

Date	Slope of probit-log lines, $b \pm s_b$	Test of parallelism χ^2 n	Assay homogeneity χ^2 n	S.D. in X , λ	Log-toxicity, $M \pm s_M$	Toxicity: nicotine/ pyrethrins	Pyrethrins $3 + \log (\text{LD}_{50})$ $\pm \text{S.E.}$
7 Feb.	3.171 ± 0.337	5.10 3	12.63 19	0.3153	{ -1.985 ± 0.069 -2.020 ± 0.069	{ 0.01036 0.00955	{ 0.718 ± 0.051 0.779 ± 0.049
8 Feb.							
3 Apr.	1.697 ± 0.354 2.107 ± 0.350	1.40 1 3.25 1	34.27 11 21.13 13	1.0398 0.6032	{ -2.737 ± 0.350 -1.804 ± 0.111	{ 0.00183 0.01571	{ 0.675 ± 0.133 0.722 ± 0.081
10 Apr.							
13 Apr.	1.549 ± 0.306	0.86 2	31.12 20	0.8049	{ -2.721 ± 0.204 -2.308 ± 0.224	{ 0.00996 0.00493	{ 0.754 ± 0.097 1.209 ± 0.153
16 Apr.							
					-2.004 ± 0.298	0.00190	1.674 ± 0.232

both the variation of the probit mortalities about the fitted lines and also the difference in slope between lines fitted individually to each poison. The assay χ^2 was well within the sampling error only in the first two tests. On 5 April ($P=0.07$) and on 10-16 April ($P=0.054$), the variability approached significance. On 3 April the variability was highly significant, although the scatter of the points indicated excessive variation among replicated treatments rather than a consistent departure from linearity. Because of the variability in three of the four assay χ^2 's, all errors have been computed with the observed error variance $s^2=\chi^2/n$, except in the tests on 7-8 February where s^2 has been assigned its expected value of 1 and allotted its observed 19 degrees of freedom ($=n$). This led to the values of $\lambda=s/b$ shown in the next column of Table 2, which were used in computing the standard errors based upon these curves.

Since the slopes for the pyrethrins and for nicotine were parallel within the sampling error for any given assay, the relative toxicity of nicotine in comparison with the pyrethrins could be represented by a single value for each test. This was determined directly as $M \pm s_M$, the logarithm of the relative toxicity and its standard error, from which the antilogarithms in the next column were obtained. The fact that the curves for the different poisons could be fitted by parallel lines met the first requirement for the hypothesis of similar action. The relative log-toxicities for the different test days, summarized in Table 2, differed from -1.80 to -2.74. Because the different determinations varied in their reliability, each has been weighted by the reciprocal of its error variance in determining a weighted mean log-toxicity. The homogeneity of the individual determinations about this weighted mean was then tested by χ^2 (Bliss, 1951) to obtain $\chi^2_M=19.05$ with 6 degrees of freedom ($P=0.004$). Even if the least reliable determination, that on 3 April, is omitted, relative toxicity varied significantly, $\chi^2_M=14.70$, $n=5$, $P=0.012$.

The variation in relative toxicity may have been due to lack of uniformity in the test preparations or in the test conditions. However, the same lots of pyrethrins and nicotine were used throughout and, as is evident from Table 2, relative toxicity did not change progressively through the test period. To relate the variation in relative toxicity to the susceptibility of the test insects, the log-LD 50's for the pyrethrins have been determined in the last column of Table 2. These median lethal doses of the pyrethrins also varied significantly, $\chi^2=20.14$, $n=6$, $P=0.003$. A scatter diagram relating log-toxicity (M) to the log-LD 50 of pyrethrins showed no relation between the two.

Thus, the toxicity of nicotine relative to that of the pyrethrins varied significantly within the same species and stock of insect and with the same type of application. Moreover, this variation in relative toxicity was not related to insect susceptibility to the pyrethrins. One criterion of similar action, that a given amount of one poison can be substituted for one unit of the other, is clearly untenable. Evidence as to the presence or absence of synergism depends, therefore, upon comparing the

mortalities actually observed in joint applications with those expected on the hypothesis of independent action. For comparison, however, the results obtained upon the hypothesis of similar action have also been included.

TOXICITY OF PYRETHRINS AND NICOTINE APPLIED JOINTLY

Nicotine and the pyrethrins were applied jointly in approximately equally toxic concentrations with the percentage kills shown in Table 3 for six different test dates. The two or four replicates in each test after those on the first two days have been combined in the table. In only two of the eleven percentages did the difference between replicates exceed the sampling error.

TABLE 3. *Toxicity of nicotine and pyrethrins applied jointly by dipping to Tribolium castaneum; observed percentage kill and percentage kill expected with different hypotheses as computed from the curves in Table 2*

Date	Concentration		Observed mortality		Mortality expected with			
	Nicotine, %	Pyrethrins, %			Independent action			Similar action
			<i>N</i>	%	<i>r</i> =1	<i>r</i> =0	<i>r</i> =-1	
7 Feb.	0.5	0.005	20	60.0	36.3	59.4	72.6	72.6
	0.25	0.0025	11	36.4	9.7	18.5	19.4	36.3
	0.125	0.00125	14	28.6	1.2	2.4	2.4	9.7
8 Feb.	0.5	0.005	14	21.4	27.5	47.4	55.0	63.7
	0.25	0.0025	15	6.7	6.1	11.8	12.2	27.4
	0.125	0.00125	15	0	0.6	1.2	1.2	6.1
5 Apr.*	1.0	0.01	34	88.2	84.1	95.6	100.0	92.7
	0.5	0.005	36	63.9	64.3	81.5	100.0	79.3
	0.25	0.0025	36	33.3†	39.5	54.6	64.4	57.2
	0.125	0.00125	34	38.2	18.4	26.2	27.9	32.5
	0.0625	0.000625	23	8.7	6.3	8.7	8.9	13.8
10 Apr.†	0.5	0.005	66	51.5	46.7	52.8	58.2	51.3
	0.125	0.00125	68	22.1	15.6	17.0	17.3	18.4
13 Apr.†	0.5	0.005	73	30.1†	21.5	29.6	31.8	30.2
	0.125	0.00125	76	9.2	4.3	5.6	5.7	7.3
16 Apr.†	0.5	0.005	73	9.6	6.6	12.8	13.2	14.8
	0.125	0.00125	70	4.3	0.7	1.5	1.5	2.4

* Each observed mortality based upon two replicates.

† Replicates differed significantly ($P < 0.02$), all other replicates agreed within the sampling error.

‡ Each observed mortality based upon four replicates.

The last four columns in Table 3 show the percentage mortality expected on four different hypotheses, by independent action at three assumed values for the correlation (r) in susceptibility and by similar action. As indicated above, the values in the last two columns are considered untenable and are given only for comparison. The mortality expected for each concentration of nicotine and of the pyrethrins has been determined from the data in Table 2 for the log-LD 50 of the pyrethrins, the M observed in the same test and the slope with which M has been computed. The

observed mortality was equal to or less than that computed for independent action with $r=0$, in eleven of the seventeen comparisons. In only one of the exceptions was the departure from expectation apparently significant statistically and here (7 February) the expected value (2.4%) fell in a range where prediction was so doubtful that the lowest dosage level was omitted in computing the dosage-mortality curve.

In one other comparison, the observed mortality seemed to be significantly less than its 'expectation' (5 April at the median concentration) but here the two replicates for the joint treatment differed significantly, which reduces the reliability of their combined value. These and all other comparisons were tested graphically with binomial probability paper on the assumption that the predictions were free of error. Since, in fact, the predicted values were themselves subject to errors of the same magnitude as the observed mortalities, there was no slightest evidence of synergism between nicotine and pyrethrins when the poisons were applied jointly.

It is of interest that in several of the higher predicted mortalities, the kill expected from the hypothesis of independent action with $r=0$ was larger than that with similar action. It is clear that Wadley's test (1945, 1949) is not a safe criterion for synergism in all instances, quite apart from the evidence for a qualitative difference in the type of action of nicotine and of the pyrethrum.

TOXICITY OF NICOTINE AND PYRETHRINS APPLIED SUCCESSIVELY

On the basis of other toxicological studies, the failure of mixtures of nicotine and the pyrethrins to show an enhanced toxicity in the present experiment was attributed provisionally to differences in rate of penetration. If the pyrethrins penetrated more rapidly, their action might have been largely neutralized within the insect before the nicotine reached the site of action. On this hypothesis, synergistic action would occur only if nicotine were followed by the pyrethrins at an interval which corresponded to their relative rates of penetration.

In the tests in which nicotine and the pyrethrins were applied successively, all of the insects were dipped twice. The application of nicotine preceded and that of the pyrethrins followed treatment with the carrier at the same time interval used between nicotine and the pyrethrins. This precaution was taken in spite of the fact that independent tests not reported here showed no significant difference between dipping in the carrier followed by the insecticide and a single dipping of insecticide.

The first two tests comparing joint and successive applications were at a single interval of 3 hr. Four or five dosage levels, forming a series of twofold dilutions, were applied in each case. Two parallel dosage-mortality lines were computed from each test in terms of the log-dose and probit mortality, one for the joint and one for successive application. The relative toxicity of successive application as compared with joint application led to the following log toxicities; on 3 April,

$M = 0.104 \pm 0.098$, and on 5 April, $M = -0.083 \pm 0.110$. Neither differed significantly from zero or equal toxicity.

The interval between applications was then varied and this time the results were positive. Except where nicotine and the pyrethrins were applied jointly for an interval of 0, nicotine was followed by the pyrethrins at intervals of $\frac{3}{4}$, $1\frac{1}{2}$, 3 and 6 hr. respectively. The tests were replicated on 10, 13 and 16 April to obtain the results summarized in Table 4. The concentrations were always in the same ratio of 100 of

TABLE 4. Multiple applications, each totalling four replicates, of nicotine and pyrethrins applied jointly (interval of 0) and of nicotine followed by pyrethrins at intervals varying from $\frac{3}{4}$ to 6 hr., showing the total number of beetles (N) and the percentage kill (%) in each case

Date	Concentration		Interval in hours between applications									
	Nicotine, %	Pyrethrins, %	0		$\frac{3}{4}$		$1\frac{1}{2}$		3		6	
			N	%	N	%	N	%	N	%	N	%
10 Apr.	0.5	0.005	66	51.5	58	91.4	64	84.4	62	71.0	53	69.8
	0.125	0.00125	68	22.1	68	51.5	58	25.9*	51	9.8	45	8.9
13 Apr.	0.5	0.005	73	30.1*	63	58.7	72	61.1*	62	58.1*	64	29.7*
	0.125	0.00125	76	9.2	68	8.8	63	17.5	55	10.9	59	8.5
16 Apr.	0.5	0.005	73	9.6	60	61.7	64	37.5	61	42.6	66	33.3
	0.125	0.00125	70	4.3	66	4.5	68	7.4	65	6.2	64	4.7

* Heterogeneity among the four replicates combined in this percentage at $P = 0.014$ to $P = 0.030$, all other percentages based upon homogeneous replicates.

nicotine to one of the pyrethrins and applied at two dosage levels, representing a fourfold difference in dose. Each treatment was applied to four lots of insects on each day, and the number dead and alive totalled over the four lots to obtain the percentage kills in Table 4. The homogeneity of the four replicates in each set was tested by χ^2 in a 2×4 contingency table. Of the thirty sets of replicates, twenty-five agreed well within the sampling error. In the remaining five, the significance of the heterogeneity varied from $P = 0.014$ to $P = 0.030$. The latter percentages have been starred in Table 4.

A dosage-mortality curve was computed from the two dosage levels for each test interval. Even though the beetles varied in susceptibility from day to day, as well as in their relative response to nicotine as compared with the pyrethrins, on any one day both relations have been considered as constant. Since the two poisons always occurred in the same proportions, comparable dosage-mortality curves could be computed on any one day for each of the five intervals between applications. Although the curves tended to have smaller slopes when the poisons were applied jointly than when they were applied at intervals, parallel lines were fitted to the data for each day. The slopes diverged significantly only on 16 April, primarily due to a small slope for the zero interval. The values of χ^2_0 comparing the slopes on a

given day have been added to those comparing replicates within treatments to obtain the assay χ^2 for each day. Since this χ^2 exceeded its expectation significantly on 10 and 13 April, all standard errors have been computed with $s^2 = \chi^2/n$.

Intervals have been compared in terms of the dose of the pyrethrins component in the mixture required to kill a constant percentage of the beetles. In order to minimize the standard error, the percentage kill on each day was the weighted mean probit for all five intervals. The results as computed initially are given in Table 5 in terms of the log-concentration of pyrethrins. Since the nicotine was

TABLE 5. *Comparison of the combined toxicities of nicotine and pyrethrins applied at different intervals to Tribolium castaneum as computed from the data in Table 4*

(The log-concentrations (+2.9) producing a constant mortality in each series are in terms of the pyrethrins component. Each has been subtracted from the weighted mean for the series and converted to its antilogarithm to obtain the relative toxicities in the last four columns.)

Date ...	Equitoxic log-concentrations (+2.9)			Relative toxicities			
	10 Apr.	13 Apr.	16 Apr.	10 Apr.	13 Apr.	16 Apr.	Combined
Interval between treatments (hr.):							
0	0.470 ± 0.065	0.563 ± 0.087	0.766 ± 0.086	0.70	0.67	0.44	0.613
$\frac{1}{2}$	0.003 ± 0.070	0.325 ± 0.082	0.239 ± 0.064	2.08	1.15	1.50	1.568
1 $\frac{1}{2}$	0.227 ± 0.066	0.227 ± 0.080	0.396 ± 0.065	1.23	1.44	1.04	1.200
3	0.427 ± 0.069	0.306 ± 0.086	0.369 ± 0.066	0.77	1.20	1.11	0.990
6	0.445 ± 0.074	0.578 ± 0.095	0.465 ± 0.067	0.74	0.64	0.89	0.777
Selected mortality (%)	50	32	24		—		—
S.D. in $X (\lambda)$	0.526	0.672	0.490		—		—

always administered in a fixed ratio to the pyrethrins, its contribution did not enter the calculation. From the standard errors for each equitoxic log-concentration (Table 5), it is evident that the amount of poison required to produce the same kill varied significantly with the interval between applications. The significance of this variation has been tested by computing χ^2 from the results on each date. In every case, the variation was significant.

The combined toxicity of nicotine and the pyrethrins not only varied significantly with the interval on each test day but the variation followed the same pattern in all three tests. The means for each interval varied significantly more than the residual variation among tests ($P < 0.025$). The relative toxicity in each interval was determined by subtracting the predicted dose for each interval from the weighted mean for the series and converting the difference to its anti-logarithm. These results are shown in the right side of Table 5, together with a weighted average for the three tests. Of the intervals tested, synergism was most pronounced at $\frac{3}{4}$ hr. and decreased thereafter until at 6 hr. it had largely disappeared and two applications were no more active than nicotine and pyrethrins given simultaneously. The toxicity of joint applications has already been shown not to exceed that expected on

the hypothesis of independent joint action. It is clear, therefore, that the toxicity of similar concentrations of nicotine and pyrethrins to *T. castaneum* by dipping could be increased twofold or more by applying the pyrethrins at a suitable interval after the nicotine.

DISCUSSION

Nicotine and the pyrethrins applied jointly by injection to *Oncopeltus fasciatus* showed evidence of synergism, but none whatever when applied to *Tribolium castaneum* by dipping. In the latter case, they acted independently. Species specificity of the poisons, which Beard (1949) had demonstrated to exist independently of route of administration, might account for these differences. However, both Beard (1949) and Menusan (1948) reported that the method of application affected the relative toxicity of two materials. Menusan showed that pyrethrum was as toxic to some insects by external application as by injection, while nicotine was much more toxic by injection. Webb (1949) was unable to increase the effectiveness of the pyrethrins in sprays with benzyl alcohol, a carrier which increased the effectiveness of diphenylamine. The toxicity of nicotine, on the other hand, can be increased by the addition of 'wetting agents', as demonstrated by Smith (1916) and many others in succeeding years.

Lindquist, Madden & Wilson (1947) found that the 'synergists' sesame oil, 'piperonyl cyclohexone' and *N*-isobutylundecylenamide applied to house-flies increased substantially the toxicity of pyrethrum applied an hour later. When applied in the reverse order, however, the pyrethrum first and the 'synergists' an hour later, toxicity was very low. They suggested that the effect of the pyrethrum on the insects was of short duration. If, in the dipping tests on *T. castaneum*, the effect of the pyrethrins was equally short-lived, and nicotine required correspondingly longer than the pyrethrins to reach a site of action, no synergism would be expected.

This reasoning led to application of nicotine followed later by the pyrethrins. When the pyrethrins were applied $\frac{3}{4}$ hr. after nicotine, the dosage required to kill 50% was about half that required for simultaneous treatment. This difference was significant statistically and represents synergism. The synergistic effect declined steadily as the time between treatments was increased, as would be expected if the insects either recovered rapidly or detoxified pyrethrins quickly. It had almost disappeared at 6 hr. The magnitude of the synergism between nicotine followed by the pyrethrins in $\frac{3}{4}$ hr. was approximately the same as estimated in the injection tests.

Although there is no direct evidence, differences between the poisons in their speed of penetrating the cuticle and reaching a site of toxic action are believed to be responsible for the relation between toxicity and the time interval between applications.

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SYSTEMIC INSECTICIDAL ACTION OF NICOTINE AND CERTAIN OTHER ORGANIC BASES

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Nicotine and nicotine salts are taken up by the roots of plants from solutions, and when 0.01–0.001 % nicotine is used the plants become toxic to *Aphis fabae* and to *Pieris brassicae* larvae and can be shown to contain nicotine. The results with *Phaedon cochleariae* adults and larvae are less satisfactory. No systemic action is observed when the nicotine is watered on to soil in which plants are growing and no nicotine can be detected in the plants. Apparently the nicotine is decomposed in the soil.

When applied several times to the upper surface of a bean leaf nicotine kills aphids on the underside. There is some evidence that nicotine can be translocated further through the plant following leaf applications, but the toxic action at any distance is very weak in the plants used in the present experiments and can only be produced by frequent applications of rather concentrated nicotine solutions. Leaf absorption and subsequent translocation has not been observed with nicotine salts.

The various organic bases, including some piperidine phosphonites and allied compounds tested, are of very little interest as contact or systemic insecticides against aphids.

INTRODUCTION

Among the more familiar insecticides which might be expected to have systemic activity nicotine is an obvious choice. It is highly toxic to insects, water soluble and at the same time a plant product and so perhaps less likely to be injurious to plants than many equally insecticidal compounds. It was with these ideas in mind that work was commenced on nicotine, and some early results with *Pieris brassicae* L. larvae were sufficiently encouraging to make a more complete investigation seem desirable. While this work was in progress a study was made of the earlier literature on systemic insecticides. It showed that as long ago as 1901 Berlese had prepared a boiling-water extract of finely chopped tobacco leaves and tested it against aphids on stinging nettles (*Urtica* spp.). The extract proved to be both more systemically insecticidal and less phytotoxic than the other materials which he tested. Nicotine in the form of solutions of 'Black leaf 40' was also used by Scherer (1927). He concluded that it was ineffective when injected into birch trees infested with the bronze birch borer (*Agriolus anxius* Gory). Davidson & Henson (1929) report that they injected bean plants with nicotine, but they give no details of concentrations used or the results obtained.

The other compounds which will be considered in this paper are mainly piperidine derivatives. Attention was given to these because they were already available and considered to be of possible interest as insecticides at a time when an arrangement

was made with the Organic Chemistry Research Laboratory at Teddington to supply candidate materials for testing as systemic insecticides. In fact, owing to staff changes only these compounds were produced under this scheme.

METHODS AND EQUIPMENT

(1) *The greenhouse*

The tests were carried out in a greenhouse maintained at about 17–23° C., and the day-length was extended where necessary to 16 hr. by fluorescent lighting. Fans were used to increase the ventilation (David & Gardiner, 1951).

(2) *The test insects and plants*

In the course of these experiments three insects have been used.

The bean aphids *Aphis fabae* Scop. were reared on broad beans *Vicia faba* L. The beans were grown in individual 'whalehide' pots 3½ in. in diameter either in John Innes pasteurized potting compost or in washed silver sand watered once a week with a nutrient solution.

The cabbage white butterflies *Pieris brassicae* L. were reared on young cabbage plants grown in pasteurized compost. The eggs and caterpillars were obtained all the year round from a laboratory culture (David & Gardiner, 1953).

The beetles *Phaedon cochleariae* Fab. were reared on young turnip plants potted in pasteurized compost according to the method to be described in a later paper.

(3) *Cages*

During the tests it is not necessary to cage the aphids on the plants, but they must be prevented from walking from one plant to the next. This can be done by placing the individual plant pots in Petri dishes surrounded by water held in flower-pot saucers (David & Gardiner, 1951).

The *Pieris* larvae were confined on the treated plants in wire-gauze cages. Small glass ring cages on a similar principle were used for the mustard beetles. These will be described in a later paper.

(4) *Assessment of the effect of the materials on the insects*

In the case of *Pieris* and *Phaedon* percentage kills were recorded. The deaths among the controls were very few and they were neglected.

The aphid population on the plants was not counted. Instead it was assessed as follows:

- Normal: Thriving colonies with aphids of all stages settled and feeding, or a few adults surrounded by young.
- Declining: Colonies with few aphids, some dead aphids on and around the plant, and the remaining aphids often moving restlessly about.
- Single: Only scattered individual aphids and no young. Many dead aphids around the plant.

Nil: No aphids on the plant.

—: Indicates that the condition referred to was passed before the first observation or not reached before the last observation.

Besides the immediate toxic effect the persistence of an effective material was also investigated. Attempts were made to re-infest treated plants, 2, 5 and 10 days after the beginning of the experiment. The toxic condition shown by the plant at the time of the re-infestation was then described as:

High: All insects killed within 2 days of re-infestation.

Moderate: All insects killed within 5 days.

Low: Some deaths but an incomplete kill in 5 days.

Nil: No effect observed.

—: Indicates that the condition referred to was passed before the first observation or not reached in the course of the test.

All the tests have been made in duplicate and the results have been confirmed by additional tests.

(5) *Assessment of the effect of the materials on the plants*

Phytotoxicity was assessed as follows:

Normal, *N*: Plant not different from the control.

Trace, *T*: Slight marginal scorch or wilt or scattered spotting.

Moderate, *M*: Large areas of the leaves scorched or pronounced wilting.

High, *H*: Plant almost entirely killed, though the small leaves around the growing point may still be green.

Potted plants may show various symptoms of ill health not attributable to treatment with the insecticides. In evaluating leaf damage the plants were therefore carefully compared with two or three untreated plants.

EXPERIMENTS WITH NICOTINE AND NICOTINE SALTS

The materials used

The nicotine solutions used in these experiments were prepared from pure re-distilled nicotine. In addition to the base, various nicotine salts, kindly prepared and supplied by Mr J. D. Campbell, have been employed. These consisted of aqueous solutions of nicotine citrate, hydrofluoride, metaphosphate and orthophosphate containing 10% nicotine.

EXPERIMENTS WITH *APHIS FABAE* ON BROAD BEANS

(1) *Contact action on aphids*

As nicotine is usually applied as a contact insecticide a few preliminary comparative tests were made and the results are presented here for reference purposes. Bean plants were used from which the tops had been cut leaving only two leaves.

These were infested with aphids and later dipped in the nicotine solutions which contained 0.1 % 'Teepol' as a wetting agent and allowed to drain and dry. The cut end of the stalk was not dipped. The temperature during the 6 days of the test varied between 17 and 23° C.

TABLE 1. *Dipping tests with Aphis fabae*

Material	Concentration (%) (as nicotine)	Aphid population. Day on which		
		Declining	Isolated	None
Nicotine	0.010	—	—	I
	0.005	—	—	I
	0.001	—	I	—
Nicotine citrate	0.010	—	—	I
	0.005	—	I	—
	0.001	I	—	—
Nicotine hydrofluoride	0.010	—	—	I
	0.005	—	I	—
	0.001	I	—	—
Nicotine metaphosphate	0.010	—	—	I
	0.005	—	—	I
	0.001	I	—	—
Nicotine orthophosphate	0.010	—	—	I
	0.005	—	I	—
	0.001	I	—	—
Control	—	—	—	—
	—	—	—	—
	—	I	—	—

From the results given in Table 1 it appears that nicotine is more effective as a contact insecticide than any of the salts tested at the same concentration of nicotine in the solution and that the salts are about equally effective. When the persistence of the nicotine was examined it was found that after 2 days the plants were no longer toxic to aphids. No leaf damage was observed at the concentrations used.

(2) *Systemic action following applications made to the roots*

(a) *Plants in culture solutions.* The bean plants were grown in sand watered periodically with culture solution. They were used when 4–8 in. high at which time they weighed 8–15 g. The test solutions were contained in half-pint milk bottles which were closed with split corks or cotton-wool to prevent the solutions exerting a direct fumigant action. It was assumed that this procedure was effective since aphids on the control plants standing among the test plants remained normal. The plants were given 100 c.c. of solution and this was renewed after 5 days.

Before extending the tests to include the nicotine salts several preliminary tests were made with nicotine. The results of one of these are given in Table 2.

The results reported in Table 2 were confirmed in several other experiments.

TABLE 2. *Nicotine in solution as a systemic insecticide, 19 October 1949*

Concentration (%)	Aphids. Day on which			Leaf damage	
	Declining	Isolated	None	Day first seen	Level on 10th day
0.05	—	1	2	7	H
0.01	2	4	5	10	T
0.005	2	7	—	—	N
0.0025	—	—	—	—	N
Control	—	—	—	—	N

They showed that the roots of bean plants absorbed nicotine in sufficient quantity from 0.01 % solutions to kill aphids feeding on the shoots of the plants. This result may be compared with bisdimethylamino phosphonous anhydride which gave a similar effect at dilutions down to 0.005 % or 0.0025 %. The margin of safety between minimum insecticidal concentration and the maximum tolerated by the plants was small with nicotine. Although these results cannot be regarded as outstanding they seemed to be of sufficient interest to make further tests with nicotine and nicotine salts worthwhile. The results of a comparative test are given in Table 3. As the plants were kept with their roots in the solutions of nicotine salts

TABLE 3. *Nicotine salts in solution as systemic insecticides, 30 December 1949*

Material	Concentration (%) as nicotine	Aphids. Day on which			Leaf damage	
		Declining	Isolated	None	Day first seen	Level on 10th day
Nicotine citrate	0.020	1	—	2	—	N
	0.010	2	3	4	—	N
	0.005	2	6	—	—	N
Nicotine hydrofluoride	0.020	1	2	3	—	N
	0.010	2	—	3	—	N
	0.005	2	5	8	—	N
Nicotine metaphosphate	0.020	1	—	2	4	M
	0.010	—	2	5	—	N
	0.005	2	3	—	—	N
Nicotine orthophosphate	0.020	1	—	2	9	T
	0.010	—	2	5	—	N
	0.005	2	5	—	—	N

for the duration of the experiment it was not possible to obtain any indication of how long the nicotine persisted in the plants at a toxic concentration. In several confirmatory tests essentially the same results were obtained.

(b) *Plants growing in sand and soil.* The plants were grown to about 6 in. high in the usual pots which held about 570 g. of moist sand or 400 g. of soil.

The required doses of the nicotine preparations were made up to 20 c.c. and poured on to the sand or soil. Subsequently, 20 c.c. of water were given to each plant daily unless more was required.

The general conclusion from this experiment is that when watered on to sand an 0.5% solution of nicotine as the base or as its salts killed all aphids on plants growing in this sand in 2-3 days and had practically killed the plants by the 10th day. The 0.05% solutions caused no obvious effects either on the aphids or the plants. When the beans were growing in potting compost no systemic action was produced by 0.5% nicotine as the free bases or as salts. With 1% solutions there was some slight action at the end of 4 days and the plants also showed damage. The reasons why nicotine only exerts a very slight systemic action from soil will be considered later in this paper.

TABLE 4. *Nicotine and nicotine salt solutions watered on sand, 3 and 14 June 1950*

Material	Concentration (%) as nicotine	Aphids. Day on which			Toxic condition of plant				Leaf damage	
		Declining Isolated None			Last day			First day Nil	First Level on seen 10th day	
		Declining	Isolated	None	High	Mod.	Low		First	Level on
Nicotine	0.50	—	2	3	5	—	—	—	5	H
	0.10	3	6	—	—	—	5	—	6	H
	0.05	—	—	—	—	—	—	2	—	N
Nicotine citrate	0.50	—	—	2	5	—	—	—	4	H
	0.10	3	7	8	—	—	5	—	6	H
	0.05	—	—	—	—	—	—	2	—	N
Nicotine hydrofluoride	0.50	—	—	2	5	—	—	—	5	H
	0.10	2	—	5	—	—	5	—	6	H
	0.05	—	—	—	—	—	—	2	—	N
Nicotine metaphosphate	0.50	—	—	2	—	5	—	—	5	H
	0.10	2	—	—	—	—	5	—	6	H
	0.05	—	—	—	—	—	—	2	—	N
Nicotine orthophosphate	0.50	—	—	2	—	—	5	—	6	M
	0.10	3	8	9	—	9	—	—	9	M
	0.05	—	—	—	—	—	—	2	—	N
Control, 4 replicates	—	—	—	—	—	—	—	—	—	—

(3) *Systemic action following applications made to the leaves*

The foregoing experiments show that nicotine can act as a systemic insecticide following absorption by the roots. It remains to be seen whether it is also systemically active when applied to the foliage of plants. Such an effect has apparently never been noticed in any experiments. But, even if it occurred, it might easily have been overlooked since any action not attributable to direct contact would probably have been ascribed to fumigation.

As pure nicotine is readily soluble in certain oils (Richter & Calfee, 1937) it should be capable of passing through the complex lipid plant cuticle. Some of the nicotine may be held by the free acids which the cuticle is reported to contain (Lee, 1925), but the remainder should reach the cell sap. It is likely that this nicotine will also be converted into salts, since the cell sap is usually acid, but in this respect it will not differ from nicotine entering the roots.

(a) *The passage of nicotine and nicotine salts from the upper to the lower surfaces of leaves*

Unless nicotine can pass through the upper surface of a leaf in sufficient quantities to kill aphids feeding on the lower surface it is unlikely to be capable of producing a systemic effect more remote from the point of application. To prove that nicotine penetrates the leaf it is necessary to ensure that the test aphids cannot reach the treated cuticle and also that they are protected from nicotine vapour passing around the leaf margin. The following arrangement was adopted to meet these requirements. A piece of bean stem bearing aphids was placed in a small glass cylinder about $1\frac{1}{2}$ in. high and $1\frac{1}{4}$ in. in internal diameter closed at the bottom with muslin. A $2\frac{1}{4}$ in. square of Perspex containing a hole 1 in. in diameter held in a retort clamp was placed on top of the cylinder and a bean leaf attached to the plant was weighted down over this hole. Next morning when the aphids had established themselves on the leaf area within the Perspex ring, the cylinder was removed and placed on top of the leaf and weighted in position with a large iron nut. The upper surface of the leaf within the cylinder was then brushed with nicotine solution through the hole in the nut. The experiments showed that solutions of nicotine, but not of nicotine salts, applied to the upper surfaces of bean leaves killed aphids feeding on the underside, immediately below the treated area. Modifications of the test method showed further that definite results could only be obtained when the treated area of the upper cuticle was larger than the area of under-surface colonized by the aphids.

TABLE 5. *Effect of nicotine preparations applied to the upper surfaces of bean leaves on aphids feeding on the under-surfaces*

Materials and details of treatments	Percentage of aphids dead on day 3
Nicotine 0.10 % v/v brushed on 8 times over 2 days	100
Nicotine 0.05 % v/v brushed on 7 times over 3 days	100
Nicotine 0.05 % v/v in capsule within cylinder for 3 days	0
Nicotine 0.10 % v/v on filter-paper disk within cylinder for 3 days	0
Nicotine citrate* 0.05 % v/v brushed on 7 times over 3 days	0
Nicotine orthophosphate* 0.05 % v/v brushed on 7 times over 3 days	0
Teepol 0.1 % v/v control brushed on 7 times over 3 days	0

* Expressed as nicotine.

As there are numerous stomata on both surfaces of bean leaves it seemed possible that the effect observed with nicotine was caused by vapour passing through the intercellular spaces. To test this suggestion nicotine solutions were placed on disks of filter-paper or in capsules within the cylinders on the upper-surfaces of the leaves. No effect on the aphids could be detected. The details concerning these experiments are set out in Table 5.

(b) *Translocation of nicotine within the plant*

Since nicotine penetrated the upper surface of a bean leaf and killed aphids feeding from the under-surface attempts were made to determine whether it was also translocated greater distances in plants following leaf applications.

In the first set of experiments in September 1950 the lower leaves of bean plants were either brushed nine times in 4 days with 0.1 % v/v nicotine solution containing 0.1 % v/v 'Teepol' or were immersed in the same solution for up to 4 days. The results were inconclusive. When 0.5 % nicotine was used in the same way the treated leaves were badly damaged. In a further experiment in March 1952 dipping the four lower leaf pairs of bean plants once in 0.5 % nicotine caused no decline in the population on the crown.

Since difficulty had previously been experienced in demonstrating translocation of insecticides following leaf applications in the broad bean (David & Gardiner, 1951) a series of experiments was conducted with young cabbage plants. The tests were set up in March 1952 at a time when the largest leaves were about 1½ in. wide. A shallow filter-paper funnel was formed around the crown, leaving four leaves below the paper on each plant. These leaves were then dipped four times daily for 4 days in 0.5 % nicotine, incorporating wetter as before. On the fifth day the treated leaves were cut off, in order to avoid a direct vapour action from them, and the remaining crown was infested with *Myzus persicae* Sul. Control plants similarly treated with 'Teepol' alone were also infested. It was observed that the *M. persicae* colonies on the nicotine-treated plants began to decline on the second day and no more than a few scattered aphids could be found on each plant by the fifth day. At this time the controls bore flourishing colonies.

It may be concluded that nicotine applied to the older leaves of young cabbages is capable of acting systemically on aphids feeding on the crown. It appears, however, that heavy and repeated doses are necessary to bring about this effect.

EXPERIMENTS WITH *PIERIS BRASSICAE* LARVAE ON CABBAGE PLANTS

Systemic action following applications made to the roots of cabbage plants

(a) *Plants in culture solutions.* For these experiments young cabbage plants have been used. The plants were removed from the potting compost, and transferred to the insecticide solutions after their roots had been washed.

As it was not known how quickly the cabbage plants would take up the nicotine preparations they were left with their roots in the solution between 1 and 5 days before the *Pieris* larvae were put on. The results of preliminary experiments of this kind are summarized in Table 6, while Table 7 shows the results of a final experiment in which the roots of the plants were in the solutions 5 days before the larvae were put on. When the larvae were put on the plants 1 day after their roots had been placed in solutions the resulting kill of larvae ranged from 0 to 100 %. This

variability doubtless reflects the different rates at which the plants took up the nicotine solutions since it disappeared when the plants had absorbed the solutions for 5 days previous to putting on the larvae. The larvae on the nicotine-treated plants consumed a small area of the leaves and then became immobilized. They remained in this state for 2-3 days, gradually shrinking in size before they finally died.

(b) *Plants growing in soil.* The cabbage plants were grown to about 6 in. high in the usual pots, holding about 400 g. of moist soil. The soil around each experimental plant was given a dose of 20 c.c. 0.1 % v/v nicotine or of one of the nicotine salts, and 5 days later ten larvae were placed on each plant. The larvae entirely consumed all the plants and also in each case a second plant treated in the same way. Obviously nicotine and nicotine salts are not readily absorbed from soil.

TABLE 6. *The systemic insecticidal action of solutions of nicotine absorbed by the roots of cabbage plants against 3rd instar Pieris larvae, 9 to 14 September 1950*

Time before larvae put on (days)	Concentration nicotine (% v/v)	Insecticidal action* (moribund and dead) (%)	Quantity of plant consumed	Phytocidal action
1	0.001	0	All	Nil
1	0.005	0	All	Nil
1	0.01	0-100†	All to very little	Nil
2	0.10	100	Very little	Slight
5	0.001	0	All	Nil
5	0.01	100	Very little	Slight

* The insecticidal action was observed on about the 5th day when either the test plant had been eaten or the larvae were dead.

† Different plants gave kills ranging from 0 to 100 %.

TABLE 7. *The systemic insecticidal action of solutions of nicotine and nicotine salts (=0.01 % v/v nicotine) absorbed by the roots of cabbage plants against 3rd instar Pieris larvae 15 April 1951*

Solution fed to roots	Percentage of larvae dead on day				
	2	3	4	5	6
Nicotine	10	20	40	100	—
Nicotine citrate	12	12	35	88	100
Nicotine hydrofluoride	0	10	30	50	100
Nicotine metaphosphate	10	10	20	90	100
Nicotine orthophosphate	0	10	33	100	—
Control	0	0	0	0	0

Systemic action following applications made to the leaves

In the experiments with *Myzus persicae* on cabbages already described, a nicotine solution was brushed on to the outer leaves of young cabbage plants and aphids placed on the inner, younger leaves were killed. During the interval between these

experiments and those about to be described, it was found that the foliage of cabbage plants taken from a greenhouse which had been fumigated in the normal way was toxic to larvae which began to feed on them as late as 24 hr. after the end of the fumigation. It therefore seemed possible that in the *M. persicae* experiment just mentioned, the unbrushed leaves had absorbed nicotine vapour given off by the treated leaves and that the nicotine had not reached them systemically through the plant. By enclosing the untreated leaves of each plant in a lamp-glass ventilated with compressed air, thereby avoiding entirely direct access of nicotine vapour, it has been shown that this alternative explanation is not true. The results obtained in several experiments with *Pieris* larvae are given in Table 8. In these experiments young cabbage plants with 7 to 8 leaves were used. The treated leaves were cut off at the end of the fourth, or the beginning of the fifth, day, and the larvae were immediately placed on the plants. Although only three of the larvae died eleven out of fifteen of those placed on treated plants made no growth for several days, they often became shrunken and motionless and showed typical symptoms of poisoning.

TABLE 8. *Effect of brushing the older leaves of cabbage plants with nicotine solution on caterpillars subsequently fed on the inner younger leaves*

(An 0.5 % nicotine solution containing 0.1 % 'Teepol' was applied for 4 days.)

Experiment no.	No. of leaves		No. of treatments per day	Day on which larvae put on	Condition of larvae after feeding on plants for 5 days		
	Treated	Untreated			N	A	D
1a	4	3	3	5	0	3	0
1b	4	2	3	5	0	0	3
2a	3	2	3	4	0	3	0
2b	3	2	3	4	3	0	0
3a	3	2	4	4	1	2	0
3b	3	3	4	4	0	3	0
Control							
4a	4	3	3	5	3	0	0
4b	3	3	4	4	3	0	0

N = normal, A = affected, D = dead. In Exp. 3 the untreated leaves were protected by the ventilated lamp-glass. Only Teepol at 0.1 % was applied to the control plants in Exp. 4. Leaves of the growing point less than half an inch across are not counted among the untreated leaves.

EXPERIMENT WITH *PHAEDON* LARVAE AND ADULTS ON TURNIP PLANTS

Systemic action following applications made to the roots of turnip plants

(a) *Plants in nicotine solutions.* Nicotine and nicotine salts gave very unsatisfactory results when tested as root-absorbed solutions against adults and larvae of the mustard beetle feeding on young turnip plants. The plants were allowed to absorb the solutions for 2 days before the insects were put on. At concentrations of 0.01 % v/v nicotine the insects were not noticeably affected and the plants remained

fairly healthy. When the solutions contained the equivalent of 0.1 % v/v nicotine they proved to be systemically active. The citrate gave the best results, killing about 40 % of the adults and larvae on the fourth day, but the plants were so seriously injured that the test could not be continued.

(b) *Plants growing in soil.* When the soil in the pots in which young turnips were growing was watered with nicotine at 0.5 % or 0.01 % v/v, and larvae were placed on the plants 48 hr. from the beginning of the treatment, they consumed the plants and grew normally.

The fate of nicotine watered on to the soil

Experiments with *Aphis* and *Pieris* have shown that nicotine can act as a systemic insecticide from solutions but not from John Innes pasteurized potting compost. In agreement with this conclusion nicotine has been found in the leaves of plants which have been kept some days with their roots in nicotine solutions but not from plants growing in nicotine-treated compost. It must, therefore, be concluded that nicotine added to soil is not absorbed by roots. Several explanations of this fact are possible: (a) the nicotine is converted into an insoluble complex such as 'nicotine peat' (Markwood, 1936), (b) the nicotine is lost from the soil by vaporization before it is absorbed by the plants, (c) the nicotine may be broken down to non-toxic materials. When these three possibilities were investigated it was found that although nicotine could be recovered from the soil immediately after treatment by steam distillation in the presence of saturated sodium hydroxide and sodium chloride, none could be recovered after 4 days. This suggests that nicotine no longer exists in the soil since it would be expected that any insoluble nicotine compounds would release free nicotine on treatment with saturated sodium hydroxide. The nicotine is also not lost by vaporization because it disappears from treated soil stored in tightly stoppered jars. It seems probable, therefore, that the nicotine molecule is decomposed in the soil either while it still exists as the free base or after it has formed salts or some insoluble complex with soil constituents. It is possible that when nicotine is added to compost it first forms insoluble compounds which cannot be absorbed by the plant and that these are subsequently more slowly decomposed.

The results obtained in the various nicotine determinations just referred to are given in Table 9. Each cabbage plant was placed with its roots in 75 c.c. of 0.01 % v/v nicotine solution or, for the experiment with soil, in 300 g. of air dry compost to which was added 80 c.c. of water and 20 c.c. of 0.1 % nicotine. Later, when sampling, the weight of the compost was made up to 400 g. by adding water before taking an aliquot. In these experiments nicotine was determined by steam distilling the sample in the presence of saturated sodium hydroxide and sodium chloride and precipitating the nicotine with silicotungstic acid. It will be noted that nicotine is readily absorbed by the roots of plants from solutions and that it appears in the leaves. At the end of 4 days no nicotine can be detected in the solution, and about

50% of the material originally present can be recovered from the stem and leaves of the plants. Some of the remainder will undoubtedly occur in the roots but, as this was not determined, it is impossible to decide whether or not some is also decomposed. The distillate from the control plants gave no precipitate with silicotungstic acid under these conditions.

TABLE 9. *Determination of nicotine in the leaves and stems of cabbage plants the roots of which had been in nicotine solution or in nicotine-treated compost and in the solutions and compost*

(In the case of plant material the nicotine is calculated on the fresh weight.)

Material assayed	Total weight taken (g.)	Nicotine recovered	
		mg./kg.	%
Six plants from 0.01 % nicotine solution after 1 day	22.5	150	—
Six plants from 0.01 % nicotine solution after 4 days	24.0	380	—
Solution in which foregoing 6 plants had been standing 4 days	50	0	0
Six plants taken from soil 24 hr. after adding nicotine	15	0	0
Six plants taken from soil 4 days after adding nicotine	21	0	0
Soil treated with nicotine in fibre pots:			
1 hr. after treatment	139	44	89
24 hr. after treatment	140	40	72
4 days after treatment	150	0	0
Soil treated with nicotine in stoppered jar 4 days after treatment	150	0	0
Control: nicotine solution 0.1 % v/v	10 c.c.	—	99

EXPERIMENTS WITH CERTAIN ORGANIC BASES AND ALLIED COMPOUNDS

The materials used

The compounds tested were prepared by the Organic Chemistry Section of the D.S.I.R. Chemical Research Laboratory at Teddington. As received, most of the samples were colourless or pale-coloured liquids, but a few were solids. The basic substances were insoluble in water, and before testing as insecticides they were converted to the acid phosphates. The neutral and alkaline phosphates proved to be insoluble. Five or six weeks after the first batch of phosphates had been prepared it was observed that most of the bases were darker in colour or had thrown down deposits. Further supplies were therefore obtained in sealed tubes. Although these resembled the original fresh samples in appearance, it proved to be impossible to form the soluble acid phosphates. No explanation can be given for this observation.

The compounds tested were: *N-n*-butyl piperidine, *N-n*-amyl piperidine, *N-n*-heptyl piperidine, *N*-cyclopentyl piperidine, *N*-benzyl piperidine, methylene dipiperidine, ethylene dipiperidine, trimethylene dipiperidine, pentamethylene

dipiperidine, decamethylene dipiperidine, *NN*₁-tetramethyl hexamethylene diamine, methyl di-*n*-octylamine, dimethyl *n*-octylamine, 2:4:6-tri (dimethylamino-methyl)-phenol, decamethonium iodide, *N*-hydroxyethyl piperidine, piperidino-*N*-acetic acid, piperidino-*N*-acetic acid hydrochloride, piperidino ethyl acetate.

Besides the above compounds a few others containing phosphorus in the molecule were prepared. These were: piperidino bis(dimethylamino) phosphonite, piperidino ethyl bis(dimethylamino) phosphonite, 2-morpholino ethyl bis(dimethylamino) phosphonite, 3-piperidino propyl bis(dimethylamino) phosphonite, 3-morpholino propyl bis(dimethylamino) phosphonite, 5-piperidino amyl bis(dimethylamino) phosphonite, 6-piperidino hexyl bis(dimethylamino) phosphonite, β -diethyl amino ethoxy bis(dimethylamino) phosphonite.

EXPERIMENTS WITH *APHIS FABAE* ON BEANS

Contact action on aphids

All the compounds were tested as contact insecticides against aphids. None of them showed high toxicity. A few of the compounds in the first list and many others allied to them have been tested previously by Richardson & Smith (1923). They concluded that none of the compounds which they tested against *Aphis rumicis* on nasturtium plants was nearly as toxic as nicotine and this conclusion certainly holds for all the compounds tested against *A. fabae*.

Systemic action with beans in solutions and in sand

In the test for systemic action the compounds were applied in solutions to the roots of broad beans infested with *A. fabae*. When tested in this way it was evident that they would be useless as systemic insecticides since any effect on the insects was always accompanied by damage to the plants. The phosphorus compounds were the most active systemic insecticides, but even the best of these was very inferior to bis(dimethylamino) phosphonous anhydride.

DISCUSSION

In some preliminary experiments the roots of broad beans infested with *Aphis fabae* and of cabbages on which *Pieris brassicae* were feeding were placed in nicotine solutions. It was found that an 0.01 % solution killed both insects, and that nicotine showed the same order of systemic insecticidal activity as bis(dimethylamino) phosphonous anhydride to aphids.

Further investigations confirmed these observations, but the interpretation is perhaps open to question. In the case of aphids on broad beans it has been found that when the roots of bean plants are placed in solutions of many chemicals the aphids become restless and wander about, but this usually only happens shortly before the plants develop marked abnormalities, such as severe wilting or scorching.

The effect in the case of nicotine and nicotine salt solutions was quite different. The aphids became restless and fell off the plants in a more or less helpless condition at dosage levels which did little or no apparent damage to the plants. They (the insects) died some time later. This is exactly the sequence of events observed with other systemic insecticides. *Pieris* larvae also showed definite symptoms of poisoning unmistakably different from those which could have been produced by starvation resulting from the refusal of the larva to feed on cabbage leaves because they had in some way become unacceptable, not because they contained nicotine, but as a result of some change induced in them by the application of nicotine at the roots. In any case, uncertainties of this kind are dispelled by the recovery of nicotine in relatively large quantities, up to 380 mg./kg. from the leaves of plants whose roots had been standing in nicotine solutions.

Nicotine also acts as a systemic insecticide when applied to the sand in which aphid-infested bean plants are growing. No such effect can be obtained, except at dosages which are phytotoxic, when the plants are growing in soil and no nicotine can be found in the plants. As far as root absorption is concerned, therefore, it appears that nicotine and its salts could only be considered as systemic insecticides for use in water or sand cultures, under which circumstances they might prove to be quite useful, especially on ornamentals where toxic residues are of little consequence.

The experiments also show that when nicotine, but not its salts, is applied to the upper surface of the leaf of a broad bean it is capable of reaching and killing aphids feeding on the lower surface. Further, it is clear that after repeated applications of relatively high concentrations (0.5 % v/v) nicotine may pass from older to younger leaves of cabbage plants and affect aphids and *Pieris* larvae feeding on them. This is true when all possibility of direct or indirect fumigant action is excluded. In the experiments described, heavy and repeated doses are necessary to produce these effects, but it seems possible that a systemic effect of this kind might also occur more readily on other plants and sometimes contribute in practice to the well-known ability of nicotine to kill aphids sheltering in curled and distorted leaves. In this connexion it is interesting to note that it has been reported (Campbell, 1949) that aphids on the growing tips of apples, roses, clematis and honeysuckle can be controlled, at least partially, by treating the lower leaves of the shoots with sprays of nicotine hydrofluoride.

We would like to thank Mr J. D. Campbell for kindly supplying samples of various nicotine salts, Dr C. F. Sharman for advice on the determination of nicotine and Mr N. J. Cartwright (since resigned) and other members of the Organic Chemistry section of the D.S.I.R. Chemical Research Laboratory in Teddington for the various organic bases and allied compounds.

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BIOASSAY SYSTEMS FOR THE PYRETHRINS

IV. STOICHIOMETRIC RELATIONS WITH PYRETHRUM SYNERGISTS*

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(With 2 Text-figures)

The sharp limitation of synergistic action when equimolecular proportions of the pyrethrins and some of their synergists are tested against flying mosquitoes has been confirmed when such mixtures are employed in assays using *Calandra granaria* L. crawling on oil-films on filter-paper.

Limitation of the relative potencies of pyrethrum-synergist mixtures appears to be a general property, and except for piperonyl butoxide the limiting relative potency is attained at equimolecular proportions of insecticide and synergist.

These observations are discussed in connexion with a general theory of insecticidal action.

INTRODUCTION

An earlier paper in this series (Page & Blackith, 1949) described the synergistic effect of the pyrethrins and two non-toxic substances, sesamin and *N-isobutylundecylenamide*. Mixtures of these substances with the pyrethrins were assayed in the form of aqueous emulsions against flying mosquitoes, *Aedes aegypti* L. It was found that for both compounds, the toxicity of the mixtures increased with addition of synergist until the pyrethrins and synergists were present in equimolecular proportions, when the mixtures were three times as toxic as the pyrethrins alone. Further increase in the proportion of the synergist did not increase this limiting relative potency which is denoted by ρ_L in this paper.

This work suggested that, for these two synergists, loose complex formation, or stoichiometric replacement of pyrethrins molecules at the site of action, might be responsible for the synergistic effects noted. The experiments on which these observations were based were repeated with a less cumbersome assay system using *Calandra granaria* crawling on filter-papers impregnated with oil-base solutions of the insecticides. The range of synergists examined was extended (Table 1).

METHODS

The twin cross-over design (Blackith, 1950) was applied to relative potency assays with mixtures of the pyrethrins and all the synergists except *N-isobutylundecylenamide*. The assays with this compound were begun before this method had been

* Part of this work was included in a thesis approved for the Ph.D. degree of the University of London.

TABLE. 1 *The parachors and limiting relative potencies of the pyrethrum synergists tested*

Compound	Molecular weight	Parachor (calculated from structure)	Limiting molecular ratio	Limiting relative potency (approximate values)
Pyrethrin I	328	738	—	—
Pyrethrin II	372	798	—	—
Cinerin I	316	730	—	—
Cinerin II	360	790	—	—
Sesamin	354	727	1:1	3.0
<i>N</i> -isobutylundecylenamide	211	602	1:1	1.8
Piperonyl butoxide	399	774	10:1	4.5
Piperonyl cyclonene	230*	530*	1:1	4.2
<i>n</i> -Propyl isome	522	800	1:1	2.3
M.G.K. 264	275	644	1:1	2.2

* Tentative values.

fully worked out, and a simple ($2+2=4$)-point assay was used for assaying the relative potencies of mixtures of this substance and the pyrethrins. In all cases the insecticides were applied in heavy white oil, diluted, to ensure spreading, with petroleum ether. In the case of '*n*-propyl isome', it was necessary to replace 10 % of this auxiliary solvent by methyl ethyl ketone, to provide a third solvent miscible with both synergist and oil. Auxiliary solvents were allowed completely to evaporate before the insects were admitted to the paper. The changes in relative potency as the proportion of synergist in these mixtures is increased are shown in Figs. 1 and 2 and require only brief explanation.

Control responses. All the synergists were tested as 2 % residual solutions, in the absence of the pyrethrins, using batches of 100 insects. Only in the case of piperonyl cyclonene were any control responses noted, and in this case only 3 % of the insects were paralysed at the end of the exposure period. This degree of paralysis was reversed during the recovery period.

RESULTS

Sesamin. For this synergist, as in the flying-insect system, the relative potency is sharply limited at the 1:1 ratio with the pyrethrins, $\rho_L = 3$ again as with flying insects.

N-isobutylundecylenamide. For this substance ρ_L is rather less than twice the potency of the insecticide alone. Because of this reduction, the limiting molecular ratio is less clearly defined, but appears to be 1:1 as in the flying-insect system. The existence of such critical values for two synergists in these widely different assay systems suggests that the stoichiometric relations between synergist and pyrethrins are important.

D.H.S. Activator (ethylene glycol ether of pinene). Pierpoint (1939) found evidence that this substance synergized pyrethrum sprays against house-flies. As

Fig. 2 shows, there is no evidence of synergistic action between the pyrethrins and D.H.S. Activator in this system, the estimates of ρ all being consistent with unity.

Piperonyl butoxide (3:4-methylene dioxy-6-propyl-5-butylbenzyl diethylene glycol ether). This synergist was examined in detail because Dove (1947) suggested

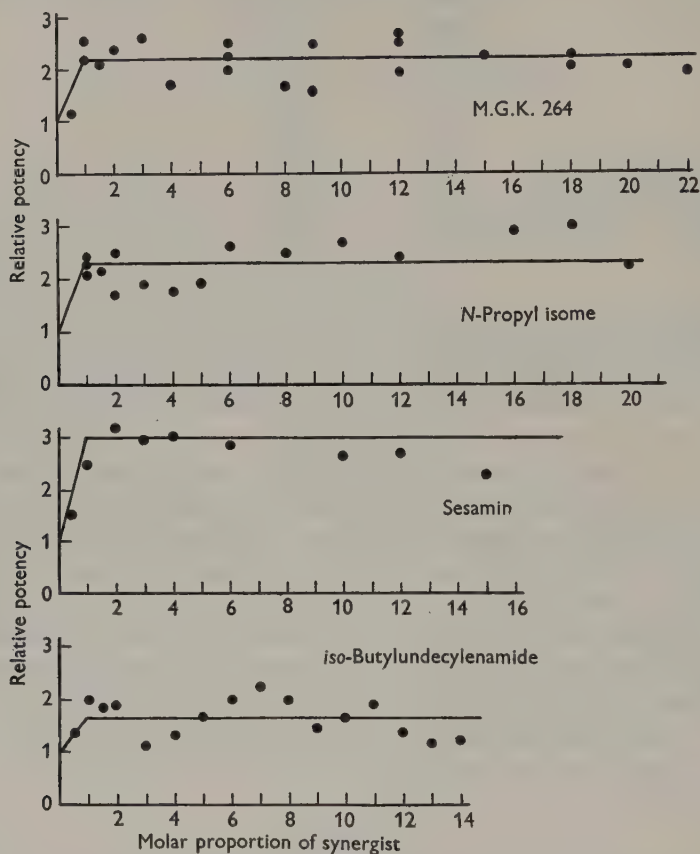


Fig. 1. Relative potencies of mixtures of the pyrethrins and synergists compared with the pyrethrins alone.

that in addition to synergism some stabilization of the pyrethrins occurred. No such effect has, however, been observed either for breakdown substantially in the dark (Hewlett, 1951) or for ultra-violet photolysis (Blackith, 1952). It seems possible that the alleged stabilization is an artifact of the method of assay used by both Dove (1947) and Chamberlain (1950) who obtained positive effects, which were interpreted as stabilization, using house-flies in contact with treated wooden panels.

As Fig. 2 indicates, ρ_L is not attained until the toxicities of the mixtures of synergist and pyrethrins have passed through a maximum of 8.0 at the 1 to 5 ratio,

falling to the limiting value of about 4.5 at the 1 to 10 ratio. The variation of the estimates of ρ_L is much greater than for mixtures having a pyrethrins:piperonyl butoxide ratio less than 1 to 5. For the comparable assays with sesamin, D.H.S. Activator, and *n*-propyl isome, there is no excessive scattering of the estimates of ρ_L . The fiducial limits of many such estimates using piperonyl butoxide have been published (Blackith, 1950). Hewlett (1951) observes that piperonyl butoxide continues to enhance the toxicity of pyrethrum films up to much higher molecular

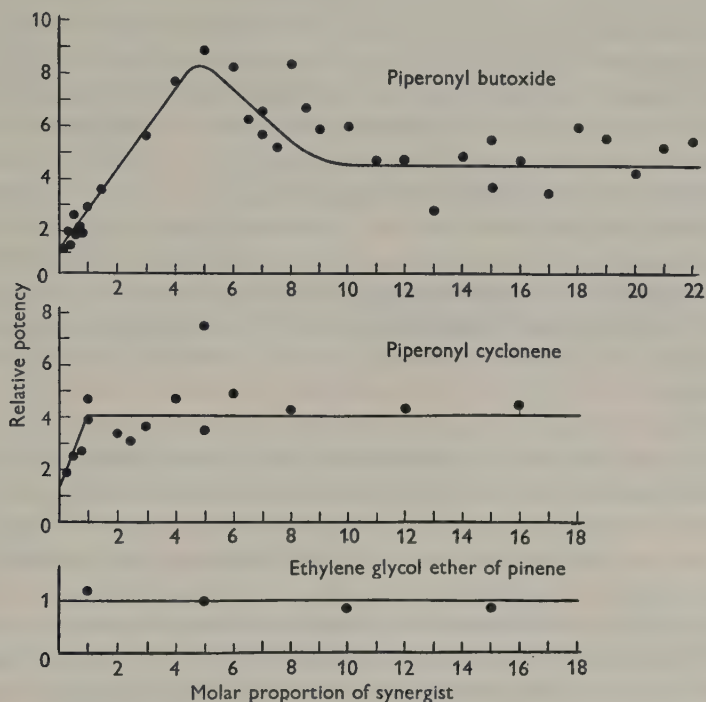


Fig. 2. Relative potencies of mixtures of the pyrethrins and synergists compared with the pyrethrins alone.

proportions than 5:1. This observation would only conflict with those recorded here if the increase in toxicity was significantly greater than that recorded in Fig. 2, namely 4.5 times that of the pyrethrins alone. An increase in excess of this value has not been demonstrated.

'*Piperonyl cyclonene*' (mixture of alkylated piperonyl *cyclohexenones*, Wachs, 1947). With this synergist ρ_L is attained at the 1:1 ratio with the pyrethrins. The value of ρ_L (4.2) is close to that for piperonyl butoxide (4.5), and the two synergists both provide estimates of ρ_L whose scatter is greater than that predicted from the fiducial ranges of the individual estimates.

'*n*-propyl isome' (*isosafrole n*-propyl maleate condensation product). Fig. 1

shows that for synergist:pyrethrins ratios greater than 1:1, ρ_L is intermediate between the values for sesamin and *N-isobutylundecylenamide* in this assay system.

M.G.K. 264 (*N*-(2-ethylhexyl)-bicyclo[2, 2, 1]-5-heptene-2, 3-dicarboximide, Hartzell, 1949). There is no important difference between the properties of this synergist and those of 'n-propyl isome' in this assay system.

DISCUSSION

Of the six synergists examined, all but piperonyl butoxide gave limiting relative potencies (ρ_L) at the 1:1 ratio, confirming the results of Page & Blackith (1949). Joly (1946) showed that stoichiometric complexes can form at interfaces when surface films of one substance are penetrated by another. Table 1 shows that one of the few features common to the synergists listed therein is a molecular volume, measured by the parachor, within the limited range of 530–800. In this range lie the parachors of the pyrethrins complex. Interfacial films may thus be formed by isosteric replacement of the pyrethrins by a stoichiometric fraction of synergist molecules. Both Welsh & Gordon (1947) and Page & Blackith (1949) have independently suggested that the pyrethrins act by discharging the resting potential of the nerves of arthropods. This resting potential probably occurs across a lipid-protein interface.

Page, Stringer & Blackith (1949) found that, at concentrations slightly higher than the minimum required to paralyse the flying insects they used, within the fixed exposure period, the weight of pyrethrins accumulated by the paralysed insects is consistently less than at the minimum concentration. This difference implies an increase in absolute toxicity of the pyrethrins, and applies to all the flying insects used in that investigation. It may be associated with the onset of close-packing at the nerve interface once the surface is covered by the insecticide molecules. The presence of synergist molecules would, by virtue of their similar volumes and capacity to form stoichiometric films with the pyrethrins, induce close-packing and its attendant increase in toxicity when fewer molecules were present than are necessary to produce the same effect in the absence of the synergist. Such close-packing would have the general property of reorienting the insecticide molecules so as to discharge the resting potential preferentially across a conjugated double-bond or other suitably mobile electronic system in the molecules. There need not be the same degree of reorientation for all synergists or in different insects so that the values of ρ_L may differ.

This hypothesis contains much that is tentative, but it provides simple explanations for the major relevant facts, accounting for the stoichiometry and partial isosterism of the pyrethrins and their synergists. Welsh & Gordon (1947) found that DDT, like the pyrethrins, probably acted by discharging the resting potential across nerve interfaces, and Kulkarni (1949) noted that the toxicity of DDT analogues increased with their dielectric constant and dipole moment. Hummer &

Kenaga (1951) have demonstrated with models the partial isosterism of DDT, rotenone, and methoxychlor, but rotenone is also synergized by many pyrethrum synergists which suggests some similarity of molecular volume between rotenone (parachor=943) and the pyrethrins (parachor=750). Probably the shape of the synergist molecule is also important, and the fact that most substances of molecular volume 530-800 are inactive as synergists indicates that this is but one factor influencing activity. All this miscellany may be generalized in the hypotheses that, among other properties needed to form an interfacial film on nerves, an insecticide should have a molecular volume lying within a limited range, and that, if such a film can be formed, molecules with a high dipole moment, at least in a preferred or induced orientation, will then discharge the resting potential. Kenaga (1950), Lord (1949) and Reimschneider (1951) have developed the first hypothesis by discussing, from various points of view, the limited range of molecular weights of useful contact insecticides. Tattersfield, quoted by West (1942), seems to have originated the second hypothesis. Together these ideas form what may be a useful guide to one aspect of insecticidal action.

The values of the limiting relative potencies discussed in this paper are not of wide importance, since their magnitude and probably their numerical order vary with the assay system employed. The sharp change of slope of the curves in Figs. 1 and 2 is critical to this discussion, however, and is an interpretation based on the fact that for all the curves (except piperonyl butoxide) the position of the change is consistent with the 1:1 molecular ratio; this hypothesis derives from Part II of this series, where a similarly placed change of slope was found in a widely different assay system. For any particular synergist, however, a best-fit curve would not warrant this interpretation exclusively.

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BIOASSAY SYSTEMS FOR THE PYRETHRINS

V. EXPERIMENTS WITH A RESISTANT STRAIN OF
CALANDRA GRANARIA L.

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(With 3 Text-figures)

Some principles of testing for resistance in wild and laboratory stocks of insects are discussed, and experiments with *Calandra granaria* L. are described in which these insects are selected for pyrethrum resistance.

A new method is described of expressing relative tolerances when the relevant regression lines are not parallel, based on the 'generalized distance' of Mahalanobis. The method may equally be used for the comparison of two insecticides giving rise to lines of different slope. A limited increase in resistance occurred on selection. Non-random oscillatory sequences of slopes were observed when the resistant strain was assayed, and regression parameters computed. The nature of these oscillations is considered.

INTRODUCTION

This paper describes an attempt to select a strain of grain weevils resistant to the pyrethrins, and to investigate the changes which occur in the bioassay system pyrethrins-oil-film-*Calandra granaria* L. when resistant insects replace the normal strains.

The relatively long life cycle of *C. granaria* (about 8-10 weeks) renders it less suitable than shorter cycle insects for genetical experiments. The emphasis here is on the effects of selection on the properties of the assay system, however, and time was sacrificed in order to use a suitable test insect. There are serious difficulties in dealing with bioassay systems involving flying insects, and of the crawling insects suitable for the pyrethrum film type of assay few have shorter life cycles than *C. granaria*.

In comparing an alleged resistant strain with populations not selected for resistance, variation within each population is no sufficient criterion of variation between populations. Intuitive judgements of the development of resistance may be based on expectations of the sampling variation within populations alone, and in some cases have apparently served only to discriminate between more and less susceptible wild populations (Brown, 1950). Blackith & Gorringer (1953) have tested the susceptibility of *C. granaria* populations from ships and warehouses to mercury vapour in the egg stage. Out of only six populations, the M.L.D. of the most resistant was at least fifty times as great as that of the least resistant. If this ratio were compared with the sampling variation within a population only, a mercury resistant strain could readily be 'discovered'.

Wild populations of insects must be suspected of genetic diversity (Harrison & Mather, 1950) and tolerance differences may not be attributable to selection. Drastic changes, where selection is known to occur, must often be associated with the spread of major genes through the population. The smaller polygenic responses to selection will generally be swamped.

The experimenter can either rely on selection acting on wild strains and confine his attention to the grosser changes in tolerance in strains already resistant, or select under laboratory conditions with the consequent expenditure of time, and the reduced probability, in the smaller populations practicable, of finding individuals homozygous for a major gene associated with resistance.

With controlled selection it is important to be able to distinguish between point events, such as mutations, and events which are 'determinate in time'. Mather & Harrison (1949) thus describe crossing-over near the centromeres, which, being induced by environmental factors, influences parallel but independent breeding units simultaneously. The distinction can be satisfactorily established only if adequate replications are available and field conditions do not meet this requirement.

METHODS

A population of adult *C. granaria* was divided into four batches of 150 insects, and four batches of 1000 insects. The larger batches were starved for 12 hr. and then tipped on to 14 cm. Whatman no. 1 filter-papers. These were impregnated with a solution of pyrethrum-in-oil as described in Part III of this series (Blackith, 1950). The concentration was adjusted to leave about 15 % of the insects not paralysed after 12 hr. exposure period. Thus batches of 150 insects were obtained which were used for starting the four parallel selected lines, and the original four unselected batches of 150 insects were used to start the corresponding unselected lines. All eight batches were mass-cultured on $\frac{1}{2}$ lb. whole English wheat in 1 lb. jam jars covered with muslin, and the old adults removed after 4 weeks. The new adults were transferred to storage jars with ample food and allowed to age for 2 more weeks. Four samples of fifty insects were drawn from each of the eight populations, and the tolerances of the selected and unselected lines estimated by a multiple ($4 + 4 = 8$)-point assay, using a technique similar to that of Blackith (1950). The concentrations used for the unselected lines were 0.17, 0.25, 0.35 and 0.50 %, equally spaced on a logarithmic scale to enable factorial coefficients to be used in calculating the regression parameters. Concentrations applied to the four resistant lines were either twice or four times these values according to the expected tolerances. Cultures for the next generation were prepared from the remaining adults. In the four selected lines, the stringency of selection was adjusted to maintain the survivors at about 150 per 1000 adults, i.e. those whose tolerances exceeded the mean by about 1 standard deviation. The first selection (giving rise to S_1) took place in December 1948, the last (giving rise to S_{15}) in September 1951. The S -notation is similar to that used by Mather & Harrison (1949) except that here $S_1 = F_1$.

RESULTS

Slopes of the regression lines

The quantal responses of samples from each population were related by the angular transformation to the logarithm of the applied dosage.

For the 120 regression lines in this experiment the three parameters representing the positions (median response dose), slopes, and residual heterogeneity were computed. Single cycle computations were done using working angles but the results of further cycles are not likely to modify any conclusions.

Fig. 1 shows the slopes from successive generations of the selected and unselected

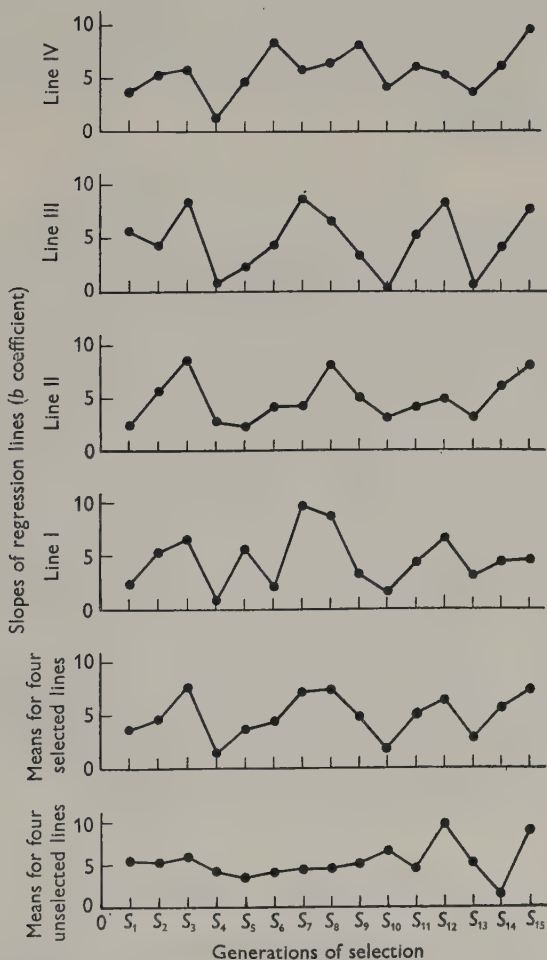


Fig. 1. Temporal variations of slopes of four independent lines of *Calandra granaria* under continuous selection, and of the mean slopes for each generation.

strains. There is a striking difference between the stability of the slopes of the regression lines for the selected and for the unselected strains. Comparison of the differences between the slopes of the four selected lines within each generation with the differences between generations within each line shows that the slopes for the four lines fluctuate in close association ($P < 0.001$). There is also a suggestion that for the resistant strain the series of slopes for each line are non-random and oscillatory. With but fifteen terms, tests for non-randomness based on turning points, etc., are liable to be insensitive (Kendall, 1946). Applied to these four series the combined probability of the hypothesis of randomness for all four lines (Fisher, 1946) is 0.06 when the observed and expected frequencies of turning points are compared, and 0.18 when phase durations are considered. These two sets of tests are not independent, and their information cannot readily be pooled, but, in conjunction with a necessarily 'intuitive' interpretation of the correlograms formed from these sequences (Kendall, 1946), one is left with a strong impression of non-randomness (Fig. 2).

Relative tolerance estimates

The expression of the influence of selection on the tolerances of the test insects is not easy. The relative tolerances of selected and unselected strains are not analogous to relative potencies, since the regression lines have in general different slopes. The usual device of taking relative tolerances at the median response dose has well-known limitations. A more cumbersome but accurate method is the expression of the set of intervals between points in terms of the 'generalized distance' of Mahalanobis (1936). This approach has much in common with a discriminant function technique.

The computation of the generalized distance begins with the construction of a matrix of the variances and covariances of the responses to doses i, j, \dots, P . Such entries in the fundamental dispersion matrix A we may denote by a_{ij} . We now require the inverse of this matrix, whose entries a^{ij} may be obtained in simple cases as the ratio: cofactor of a_{ij} in A /determinant $|A|$. When more than three doses are used, this method is tedious, and the general method is recommended. The dispersion matrix A is made the left-hand side of a set of simultaneous linear equations having as right-hand side the symmetric matrix formed by setting the covariances at zero, and the leading diagonal of the variances at unity (Fisher, 1946). The entries in the inverse matrix are the solutions of this set of equations, expeditiously found by the Doolittle technique (Dwyer, 1941).

The generalized distance d is given by the expression

$$Pd^2 = \sum_{i,j=0}^{i,j=P} a^{ij}(a_i^s - a_i^t)(a_j^s - a_j^t),$$

where a^s and a^t are the responses of the standard and test populations of insects to the doses i, j, \dots, P . Such responses are conveniently computed in working angular units; with this finite distribution the area between the two regression lines is

completely bounded. This area, or, where the regression lines cross within the experimentally attained range of responses, the difference between the two parts of the area, is fully specified by the constant range of the distribution and the estimate of d in any particular case. The generalized distance is computed as a dependent variate difference, and generalizes Finney's (1952) 'mean probit difference', in respect of the differential weighting of, and correlation between, the variate differences. If, as here, one strain is regarded as a standard, the slope of its regression line can be used to translate the generalized distance into the logarithmic dose metameter, from which follows a quantity with a claim to be considered as a generalized relative tolerance or, for comparisons between insecticides, generalized relative potency.

This generalized relative tolerance of a test population, with respect to a standard (or generalized relative potency in the case of a comparison between insecticides) may be defined as the length of the join, parallel to, and measured in the same units as, the dosage metameter axis, between the observed regression line deriving from the 'test' and that line of equal slope for which the d^2 comparison with the 'standard' regression line is a minimum.

The dosage intervals should be equal on a logarithmic scale, and, if the doses applied to the standard population have to be different from those applied to the test, because of a wide difference of susceptibilities, the common difference between the two sets of doses is added to the estimate of relative tolerance. The measure has the advantage of estimating the relative tolerance over that part of the response range attainable in practice, without the necessity for extrapolating to median response doses, which in the extreme case of a regression line of low responses and low, but significant, slope, may lead to physically absurd estimates.

Expressed in this manner the differences attributable to selection are shown in Fig. 3 and compared with representation by median response dose ratios. The improved stability of the new measure is evident. The relative resistance of the selected lines did not increase until S_6 , and, after rising to a value of about 3.5 remained at that level under continuous selection. This increased tolerance is of the same magnitude as that found by Weiner & Crow (1951) selecting *Drosophila melanogaster* (Meigen) for resistance to DDT, and by Pielou & Glasser (1952) who selected *Macrocentrus ancylivorus* (Roh.) against the same insecticide. Agreement between the generalized distances and median response dose ratios seems likely to be closer in these experiments than may be anticipated in other work, since the resistant strain has regression lines which tend to pivot about a relatively stable median response dose as the slopes oscillate.

Measurements of relative tolerance or potency by means of the generalized distance will not generally agree when different dosage levels are used in otherwise comparable experiments, but such discrepancies are a feature of the divergence of the regression lines being compared. It is important to distinguish between the limitations of any particular method of measuring relative tolerances, and the

limited usefulness of the idea of generalized relative tolerance; no more information is to be expected from the use of the generalized distance than is inherent in the comparison being attempted.

DISCUSSIONS AND CONCLUSIONS

Selection for resistance to paralysis by the pyrethrins can uncover limited heritable differences among the weevils used. These differences are less marked and are discriminated only by longer selection than their reactions to light where two

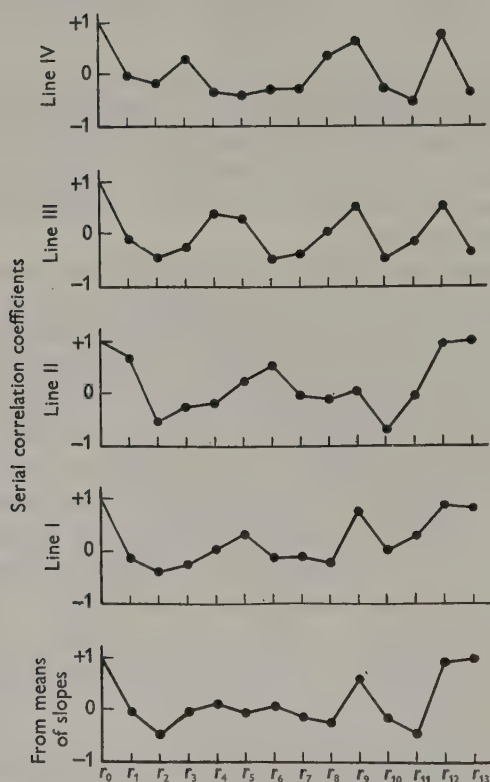


Fig. 2. Correlograms formed from serial correlations of slopes from selected lines detailed in Fig. 1.

genetic types were involved (Richards, 1951). The resistant lines showed no significantly enhanced cross-resistance to DDT when assayed against this insecticide. In differing in this respect from the many instances of gross irreversible increases in resistance reported with chlorinated hydrocarbon insecticides, these experiments emphasize that wide generalization about the mechanism of resistance is of uncertain value.

The suggestion from Fig. 3 that the change in tolerance is concentrated immediately before generation S_8 is supported by examination of the χ^2 residuals for the regression lines fitted to the data from the resistant lines. Out of the fifteen selected generations, only S_5 , S_6 and S_7 have more than one of the four χ^2 residuals significantly in excess of expectation at the 5 % level. For these three generations three of the four residues are excessively high in each case, indicating departure

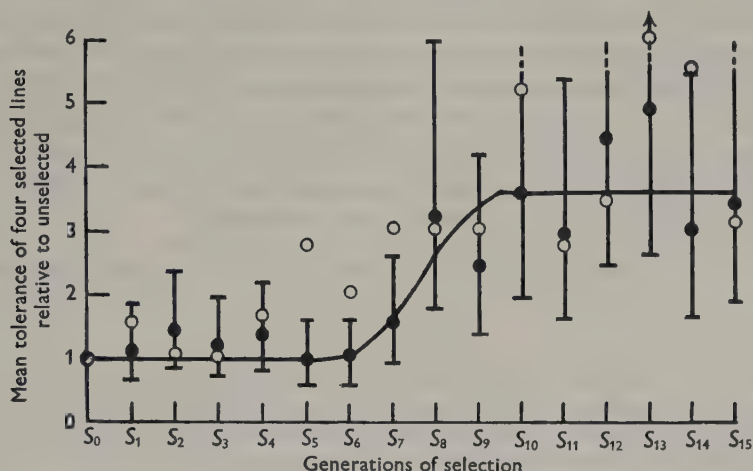


Fig. 3. Development of a strain of *Calandra granaria* resistant to pyrethrins. —○—○—, relative tolerances at median response doses; —●—●—, relative tolerances computed from 'generalized distance' between the regression lines (with approximate 95 % fiducial ranges).

from linearity, and the median response dose ratios lie outside the 95 % fiducial limits of the generalized distance. Thompson (1950) has pointed out that truncation of the low-resistance end of a distribution will raise not only the mean but the slope of derived regression lines. During this transition, curvature is expected in these lines, though the overall change of slope will be masked by the oscillatory changes.

For the first eleven generations, while the cultures were kept in an incubator with a high temperature differential, the slopes from the unselected strain lay within normal limits, and only began to oscillate after the cultures had been transferred to a constant temperature room with a negligible differential. The period covered by the first eleven generations was over 2 years, and the change from a stable to an oscillating slope at this time can hardly be fortuitous. The selected strain gave regression lines the slopes of which oscillated from the beginning of the experiment, and the oscillations continued when selection was relaxed on the samples of each line from S_6 for six further generations showing that continued selection is not a prerequisite for such oscillations.

Apart from the frequency of the oscillations, mutations as a cause are rendered

improbable by the closely similar behaviour of all four independent lines in the selected strain. This fact suggests strongly that the immediate cause of the oscillations is determinate in time and not a point-event. Further, their occurrence in apparently non-random sequences suggests some repetitive process acting over several generations, this view being supported by the continuation of the oscillations in samples of each selected line allowed to breed without selection for a year from S_6 onwards. Richards (1948) obtained by selection a line of *Calandra granaria* whose weights in successive generations fluctuated about a mean partly as a result of the opposing effects of selection for weight and infertility. No reduction in the productivity of the cultures used for the work reported here could be detected. The commencement of oscillation in the unselected strain, after so long a period of stability and associated with at the most a relatively mild environmental change, indicates that for the other, selected, strain selection is either incidental to the oscillations, or is one of several possible 'trigger' events.

I am grateful to Miss M. Roberts for assistance in computing the generalized distances.

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STUDIES ON BEETLES OF THE FAMILY PTINIDAE

VIII. THE INTRINSIC RATE OF INCREASE OF SOME
PTINID BEETLES

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The results of laboratory studies on nine species of warehouse Ptinid beetles at 25° C. and 70% R.H. are used to calculate by Leslie's methods, the statistic r measuring the intrinsic rate of increase of each species. *Ptinus tectus* Boield. has the highest value of $r = 0.38$, equivalent to a 39% weekly increase or tenfold in under 2 months. Four species, *Gibbium psylloides* (Czemp.), *Mezium affine* Boield., *Stethomezium squamosum* Hinton and *Trigonogenius globulus* Sol., are capable of tenfold increase in 3 months and the remaining four multiply more slowly, either because of a low oviposition rate (*Eurostus*), high developmental mortality (*Niptus*), or slow development (*Ptinus fur* (L.), *P. sexpunctatus* Panz.).

Generally the intrinsic rate of increase falls with temperature due to lengthening of development and reduction of oviposition, but near the maximum of development it also falls because of high developmental mortality. *Niptus* has a higher rate of increase at 20° C. than at 25° C. which is close to its developmental maximum. Relative humidity has little effect except at the limits of humidity tolerance, but water must be occasionally available for the adult to drink, or oviposition and thus rate of increase is markedly depressed. Food mainly affects the rate of increase through its effect on developmental speed. Other factors are briefly considered.

The statistic r applies to a population which has a stable age distribution. For Ptinids the stable age population was found by calculation to contain between 5 and 11% of adults, whereas in the warehouse never less than 15% of the population found were adults. In a warehouse actual observed rates of increase reached a value equivalent to $r = 0.24$ per week over a period of 8 weeks. From observed temperature means and laboratory results a maximum of $r = 0.31$ was predicted with a mean of 0.19 per week for a year. The value of r obtained using the oviposition rate estimated in the warehouse was 0.1. Warehouse values of r may be below those predicted from laboratory results because oviposition is restricted due to lack of drinking water and often to overcrowding, mortality increased due to disease, parasites, predators, and accidental cannibalism, and developmental speed retarded due to crowding.

INTRODUCTION

One of the main objects of investigating the biology of insect pests of economic importance is to determine how rapidly the insect population will grow. It is usual to make subjective estimates of the likelihood of serious outbreaks from the information obtained. The figures obtained normally include the period required for the development of the species on particular foods, the mortality at each stage of life and the rate of egg laying. Recently, Leslie & Ranson (1940) pointed out that the methods of human demography could be applied to the populations of mammals, and Birch (1948) and Leslie & Park (1949) each applied these methods to an insect pest of stored foodstuffs.

In this paper, the same methods are applied to a number of beetles of the family Ptinidae, all of which are warehouse insects, although only one is a serious economic problem. The results used have been published by Howe & Burges (1951, 1952, 1953a) and are here summarized in Tables 1-3. The statistics calculated are the intrinsic rate of increase r and the finite weekly rate of increase λ , theoretical statistics which give the maximum rates of increase possible for a population with a stable age distribution, if the basic results used are reliable. The most obvious factor which is generally not available is the density effect of crowding, but in general, apart from some considerations discussed later, it is doubtful if the effects of overcrowding are important until outbreak limits are exceeded.

EXPERIMENTAL RESULTS

For all species tables of the oviposition rate were obtained using isolated pairs of beetles in 2×1 in. glass tubes with wheatfeed plus 5 % by weight of yeast as a food and oviposition medium. To facilitate the removal of eggs, this food was ground to pass a sieve of 60 meshes to the inch. Food was generally renewed fortnightly. Eggs were removed by sieving usually three times a week, and at the same time the adult beetles were given drinking water on damp cotton-wool. The rate of oviposition of *Ptinus tectus* in more crowded conditions was also determined. At each of several temperatures four groups of fifty adults were placed on food about $\frac{1}{4}$ in. deep in 1 lb. jam jars. Dead adults were removed and sexed at each sieving, and when the number of live insects in any of the jars fell to about forty, the beetles from one jar were shared among the remaining three in such a way that the new density did not exceed sixty in any jar. Similar adjustments were made again later by reducing the experiment to two and then to one jar at each temperature.

Tables 1 and 2 express the egg number in terms of eggs per female alive at the beginning of the experiment, hence no adjustment is necessary to allow for death of the female adults during the experiment. Some adjustments have been made for a few females lost during the experiments.

The pre-adult death-rate and development periods are taken from experiments in which larvae were grown on wheatfeed when isolated in $2 \times \frac{1}{2}$ in. glass tubes (Table 3).

For most species information on the rate of oviposition is available only for 25° C. 70 % R.H., and hence the statistics are calculated only for this temperature, but for *P. tectus* oviposition rates were obtained over a wide range of temperature and the effect of temperature on the statistic r is examined.

THE CALCULATED STATISTICS

The methods of calculation are given in detail by Birch (1948) and briefly by Leslie & Park (1949), and since they can be applied quite simply to the figures in the form presented in Tables 1-3, the calculation details are not repeated here. Table 4 gives the values calculated for r and λ assuming the sex ratio of offspring to be unity. This assumption appears valid for Ptinids.

TABLE 1. *Egg number per female of several Ptinid species kept as isolated pairs at 25° C., and of Niptus hololeucus at 20° C.*

(The figures are based on the number of females alive at the beginning of the experiment. R.H. 70 %)

Week	<i>Eurostus</i>	<i>Gibbium</i>	<i>Mezium</i>	<i>Niptus</i> , <i>Niptus</i> ,		<i>P. sex-</i> <i>P. fur punctatus</i>	<i>P. sex-</i> <i>punctatus</i>	<i>Stetho-</i> <i>mezium</i>	<i>Trigono-</i> <i>genius</i>	<i>P. sexpuncta-</i> <i>tus (modified</i> <i>arrangement)</i>
				25° C.	20° C.					
1	0.4	9.7	2.2	3.3	3.5	1.3	0.3	3.1	14.2	0.03
2	0.2	12.0	3.1	8.0	12.0	1.1	1.2	4.0	7.9	0.1
3	0.5	10.2	10.2	7.6	17.5	3.8	3.8	7.4	10.7	0.5
4	0.2	9.8	9.9	5.4	16.3	6.3	5.9	6.2	8.8	1.0
5	1.0	12.5	10.5	4.7	11.3	5.4	2.7	7.9	7.0	1.3
6	1.1	11.9	9.4	4.7	21.5	5.7	1.8	9.3	6.9	1.4
7	1.0	7.6	9.3	2.6	12.5	4.6	0.6	6.0	3.4	1.3
8	1.3	9.8	8.1	1.5	2.0	2.7	0.3	6.8	4.6	1.0
9	1.3	11.5	10.2	1.1	9.0	3.4	0.1	6.4	4.7	0.5
10	1.1	7.8	11.5	1.3	7.4	2.7	0.8	5.4	2.8	0.3
11-15	2.1	43.1	47.1	3.4	25.1	1.6	3.1	30.8	20.4	1.2
16-20	0.5	40.2	47.5	1.9	14.0	0.04	0.3	23.3	24.8	4.9
21-25	—	33.9	41.3	0.8	—	—	0.5	17.1	19.6	4.8
26-30	—	32.3	32.7	—	—	—	0.2	14.8	17.7	1.9
31-40	—	21.8	64.7	—	—	—	—	22.4	10.7	1.1
41-50	—	4.4	74.2	—	—	—	—	—	1.7	0.2
51-60	—	—	52.1	—	—	—	—	—	—	—
61-75	—	—	14.6	—	—	—	—	—	—	—
Total	10.7	283.1	458.2	46.2	152.0	38.6	21.3	168.8	161.7	21.3
No. of females	10	34	13	20	2	27	30	24	24	30

TABLE 2. *Egg number per female of Ptinus tectus at various temperatures*

(Figures are based on number of females present at beginning of experiment. R.H. 70 %.)

Week	Tested as isolated pairs				Tested in groups of 50				
	15° C.	20° C.	23° C.	25° C.	13° C.	20° C.	25° C.	27° C.	30° C.
1	10.8	15.9	16.3	28.3	2.4	18.6	17.0	19.4	12.7
2	18.8	22.0	22.7	33.4	3.5	14.6	18.7	19.0	7.5
3	16.5	19.1	31.7	34.5	1.9	11.8	19.4	18.2	9.8
4	16.8	20.6	27.1	31.2	1.1	12.5	16.9	18.2	10.0
5	10.0	19.5	28.9	30.6	0.4	11.9	13.2	17.8	9.2
6	8.8	18.4	25.8	28.3	0.5	10.3	10.7	16.0	5.4
7	2.5	15.4	24.2	26.0	0.7	9.3	10.8	13.4	3.3
8	5.5	12.3	20.2	26.0	0.5	7.7	7.5	10.6	1.5
9	7.8	8.9	23.2	22.9	2.2	8.8	8.1	13.2	1.2
10	6.0	Stopped	22.8	20.9	2.5	13.3	12.4	13.0	0.3
11-15	29.1	—	99.8	71.7	15.3	56.2	46.4	42.9	2.7
16-20	40.6	—	79.9	18.2	7.3	48.6	32.3	32.8	—
(2 weeks)									
21-25	27.4	—	Stopped	Stopped	4.3	34.2	17.0	10.1	—
26-30	18.6	—	—	—	10.7	47.5	24.3	16.1	—
(3 weeks)									
31-40	Stopped	—	—	—	9.1	72.7	18.1	—	—
41-50	—	—	—	—	3.1	37.0	3.6	—	—
51-60	—	—	—	—	8.8	5.5	—	—	—
61-75	—	—	—	—	1.9	—	—	—	—
Total no. of females	4	20	17	19	100	114	128	128	113

TABLE 3. *Additional information used in calculation of r and λ*

(Slightly modified from Howe & Burges, 1951, 1952, 1953 a, b)

Species	Temp. ($^{\circ}$ C.)	Percentage eggs hatching	Percentage larvae becoming mature adult	Total percentage eggs becoming \varnothing adult (assuming 1 \varnothing :1 σ)	Development period, to middle of week in which first egg laid
<i>Eurostus</i>	25	73	80	29	9
<i>Gibbium</i>	25	72	76	27	11
<i>Mezium</i>	25	58	48	14	13
<i>Niptus</i>	25	82	13	.5	15
<i>Niptus</i>	20	75	79	30	17
<i>P. fur</i>	25	75	75	28	21
<i>P. sexpunctatus</i>	25	75	50	19	27
<i>Stethomezium</i>	25	54	88	24	12
<i>Trigonogenius</i>	25	76	80	30	11
<i>P. tectus</i>	30	31	0	0	—
<i>P. tectus</i>	27	50	76	19	9
<i>P. tectus</i>	25	90	92	42	9
<i>P. tectus</i>	23	90	90	41	10
<i>P. tectus</i>	20	90	88	40	12
<i>P. tectus</i>	15	95	86	42	21
<i>P. tectus</i>	13	95	84	40	25.5

TABLE 4. *Statistics calculated from experimental results using wheatfeed as food*

Species	Value of r per week	Value of λ per week	Net reproduction rate, R_0	Mean length of generation, weeks, T
<i>Eurostus</i>	0.072	1.075	3.1	15.7
<i>Gibbium</i>	0.235	1.265	76.4	18.5
<i>Mezium</i>	0.160	1.173	64.9	26.1
<i>Niptus</i> , 25 $^{\circ}$ C.	0.043	1.044	2.3	19.5
<i>Niptus</i> , 20 $^{\circ}$ C.	0.175	1.191	46.8	22.0
<i>P. fur</i>	0.094	1.099	11.1	25.6
<i>P. sexpunctatus</i>	0.044	1.045	4.1	32.1
<i>Stethomezium</i>	0.178	1.195	42.1	21.0
<i>Trigonogenius</i>	0.227	1.255	48.1	17.1
<i>P. tectus</i> , 15 $^{\circ}$ C.	0.15	1.162	—	—
<i>P. tectus</i> , 20 $^{\circ}$ C.	0.28	1.323	—	—
<i>P. tectus</i> , 23 $^{\circ}$ C.	0.355	1.377	—	—
<i>P. tectus</i> , 25 $^{\circ}$ C.	0.395	1.462	—	—
<i>P. tectus</i> groups:				
13 $^{\circ}$ C.	0.084	1.088	23.3	37.5
20 $^{\circ}$ C.	0.262	1.295	168.2	19.4
25 $^{\circ}$ C.	0.344	1.394	122.7	14.5
27 $^{\circ}$ C.	0.286	1.331	49.5	13.6
30 $^{\circ}$ C.	0	—	—	—

 R_0 is the total number of adult female offspring produced by a single female. T is a statistic calculated as $\log_e R_0/r$.

The values given apply only to populations with a stable age distribution, so it is necessary to examine the structure of such a population. Development mortality is similar for all species, usually fairly high for the egg (Table 3), sometimes high for the very young larvae and very low for older larvae, pupae and immature adults. Fuller details are given by Howe & Burges (*P. tectus*, 1953*a*; *P. fur* and *P. sexpunctatus*, 1951; *Mezium affine*, 1953*b*; and other species, 1952). The general picture for Ptinids thus closely resembles that given for *Calandra oryzae* L. by Birch (1948), except that the adults live longer and maintain a more even rate of oviposition. It is not surprising, therefore, to get a very similar age distribution. Thus for *P. tectus* at 20° C., the stable age population contains between 5 and 6 % of free adults, and a similar proportion of pupae and adults in the larval cocoon. About one-third of the population is in the egg stage. Although adults can live and oviposit for more than 1 year, under 2 % of a stable population would be adults older than 1 month. At 13° C., about 11 % of the stable age population are free adults.

In the warehouse, it is very difficult to measure age distribution since larvae are not easily found, and in any event the adult and pre-adult stages may occupy different niches of the environment. Thus *Ptinus* larvae feed mainly in the outer layers of sacked produce, but most of the adults wander freely outside of the sacks. Even so, Howe (1950) records that adults comprised 15 % of a population inside sacks exposed to *P. tectus* infestation for 9 months. It is possible that some of these emerged from cocoons at the time of examination or were unable to escape from the sacks. In the fabric of a warehouse Howe estimated there were of the order of 10,000 large larvae and pupae and a similar number of larvae in flour dust and spillage. The number of adults present may have been as large as 150,000, and though it may be admitted that they are more obvious because more active, it seems unlikely that an even greater number of larvae were present in pockets of food hidden away in crannies in the fabric of the building.

It is probable, therefore, that a true warehouse population contains a larger proportion of adults than a stable age population, with the inference that the rate of increase is below the maximum possible. The most likely reasons for this are that in the field drinking water is less easily available than in the laboratory experiments so that egg production is slower, and the death-rate of all stages much greater. Again, light depresses activity and may reduce egg laying in some lighter warehouses.

It is of interest to compare Birch's figure of 5 % adult *C. oryzae* in a stable age population with those recorded by Howe (1943) for an infested bin of wheat. At the peak of this infestation adults formed 11 % of the population; 9 weeks later when larvae were unable to escape from the region of unfavourably high temperature they had induced, but the more mobile adults were able to move away, the proportion of adults rose to 14 %. The age distribution of immature stages at the earlier date shows the expected preponderance of the younger stages. These data can be considered more reliable than field observations on *Ptinus*.

In spite of these reservations the statistic r may reasonably be used to compare the maximum rates of increase of species of similar habits. *P. tectus*, the only species which is important in Britain, has the highest value of r , and the corresponding value of λ shows that this species is capable of increasing by 39 % per week, that is, doubling its numbers in little more than 2 weeks and of increasing ten times in just under 2 months. High values of r at 25° C. are also given by four species known to be able to survive at 30° C. (Howe, 1949*a*), *Gibbium psylloides* (Czemp.), *Trigonogenius globulus* Sol., *Stethomezium squamosum* Hinton, and *Mezium affine* Boield. All of these are capable of increasing by ten times in 3 months, but in fact none has ever been recorded as serious pests in Britain, and it may be concluded that they have never been left undisturbed in favourable conditions of food and climate. The remaining four species for which figures are available breed slowly at 25° C. *Eurostus hilleri* (Reitt.) does not oviposit well in the laboratory, but this may reflect unsuitable culture conditions. If the figure obtained here is reliable then the species would require 10 weeks at 25° C. to double its numbers. In the 13 years since it was first recorded in Britain (Howe, 1940), it has spread throughout the country, but nowhere is it numerous. *Niptus hololeucus* (Fald.) is very close to its maximum at 25° C. Howe & Burges (1952), tested two groups of ovipositing adults. One did not lay enough eggs to compensate for the heavy larval mortality at this temperature. The other group laid better but gave a lower rate of increase than *Eurostus hilleri*. No developmental data are available at 25° C. for two remaining species, but estimates of developmental periods and mortality are made from results at the closest temperatures available. *Ptinus fur* (L.) is liable to undergo diapause but that phenomenon has been ignored here because all the diapausing individuals in experiments at 23° C. were male (Howe & Burges, 1951). This species can multiply threefold in less than 3 months. The comparatively slow rate of increase is mainly due to slow development. *P. sexpunctatus* Panz. also increases slowly. Information on this species is given for 20 and 30° C., and at both temperatures the adult of this species rests in the cocoon for a period at least as long as the period of development from laying to adult. If the adult were assisted to emerge from the cocoon by some outside agency as it might possibly be in its natural habitat of bees and birds' nests, it would be able to multiply much more quickly. The small egg output of the last two species also restricts their rate of increase. Braune (1948) states *P. latro* F. and *P. hirtellus* Stm. closely resemble *P. fur* and *P. sexpunctatus* in their ecology.

THE EFFECT OF SEVERAL ENVIRONMENTAL FACTORS ON ' r '

(a) Temperature

The effect of the external environment on the statistic r may operate through all of the main components of this factor, speed of development, mortality and rate of oviposition. Unfortunately, only for *P. tectus* are data for all three of these factors

available for several different combinations of conditions. In particular, egg-laying rates for most species have been determined only at 25° C.

An estimate of r is made for *Niptus* at 20° C., using an estimate of oviposition based on two females only. This shows *Niptus* to multiply quite rapidly at this temperature at which larval mortality is considerably less than at 25° C. An ability to multiply itself ten times in 6 months is sufficient to account for the occasional local outbreaks which have been recorded from time to time in Britain and Germany.

A wide range of temperature data is available for *Ptinus tectus* which can be regarded as typical of the group in its reaction to temperature change. Oviposition may occur at 30° C., but this is too high for larval development so the population dies out at this temperature. Very rapid egg laying also occurs at 27° C., being higher than at 25° C. for just over 10 weeks. Eggs laid early in life are much more effective in population increase, so even though 27° C. is near the maximum for the species there is an impetus for rapid population growth. Development is only slightly slower, and the major factor in reducing the rate of population growth at 27° as compared with 25° C. is the high mortality of eggs and young larvae, the older stages being able to grow safely.

At 23 and 20° C. the values of r are successively lower than at 25° C., both oviposition and developmental rates being lower but the values are still higher than for the other species at 25° C. Further falls of r are apparent with fall of temperature, but at 15 and 13° C. r is high enough to indicate a two- to three-fold increase in 3 months.

In general, these results confirm the conclusions of Birch (1948) that the main effect of temperature on r is caused by the lengthening of the developmental cycle at lower temperatures. There is little change in mortality except at limiting temperatures, and although 50 % more eggs are laid at 20° C. than at 25° C. (420 as against 275), they are laid more slowly early in life at the lower temperatures. Thus, except at limiting conditions, comparison of the periods required for development should give a reasonable guide to the effect of these conditions on the rate of increase. *Gibbium psylloides*, for instance, may be expected to increase faster at 33 and 30° C. than at 25° C. because the development periods are about 7 and 8 weeks respectively instead of 11 weeks. Similarly, all species except *Niptus* increase more slowly at 20° C. than at 25° C. because development periods are 2-7 weeks longer.

(b) Relative humidity

Relative humidity has a comparatively slight effect on the speed of development except at the lower tolerance limits, and only these low humidities have any appreciable effects on mortality. The oviposition rate of groups of adults at relative humidities of 80, 70 and 50 % are compared in one experiment, but it is somewhat unreliable in that the sex ratio in the groups was not determined but was assumed to be unity. There was no very great difference in egg laying, and accordingly no

marked effect on r is expected. The value of r for 50 % R.H. is about 0.03 lower due to the extra week required for development.

The availability of free water for the adults to drink plays a great part in the size of r . In the experiments on oviposition at 13° C., one set of freshly emerged adults was denied drinking water, and as a result failed to lay enough eggs to maintain the population. Laying stopped in 3 weeks, and in all only about 90 eggs per 100 beetles were produced. At 23° C. a similar experiment was performed, except that all beetles were given adequate water at the start of the experiment. Enough eggs were laid during the first 2 days to allow population increase, but after that the oviposition rate was low. It is evident, however, that in practice occasional drinks will enable *Ptinus tectus* to lay enough eggs to increase in numbers. A similar conclusion was drawn from an experiment done in an unheated building (see p. 130). As all other warehouse Ptinids also require drinking water and have a lower potential rate of increase, it seems reasonable to conclude that they may need more frequent drinks to lay enough eggs to ensure a population increase.

(c) Food

Food must affect r by its influence on the speed of development. For all the warehouse Ptinids considered here development is quicker at 25° C. on wheatfeed than on flour, usually by about a week. On fishmeal, development may be slower than on flour by less than a week, e.g. *Gibbium*, or by much more, e.g. *Trigonogenius*—8 weeks. These differences are sufficient to reduce the value of r on fishmeal by about 0.01 for *Gibbium* and about 0.06 for *Trigonogenius*. Developmental mortality increases considerably on some foods, notably fishmeal. *Eurostus* fails completely on this food and the death-rate of *Trigonogenius* is very high. Doubling mortality reduces the value of r by about one-fifth for Ptinid beetles, and other species which lay moderately well for a long time.

There is little precise information on oviposition rates in different foods. In short-term experiments, both *Ptinus tectus* and *Eurostus* when confined on particular foods laid much better on fishmeal than on wheatfeed, with intermediate results on flour. Given a choice of foods in which to oviposit, *Ptinus fur* and *Stethomezium* generally preferred fishmeal and *Eurostus*, *Niptus*, *Trigonogenius* and *Ptinus sexpunctatus* showed some preference for cereal derivatives. Preference for fishmeal, or a greater readiness to lay in it, could to some extent promote more rapid increase in fishmeal than in wheatfeed.

Two further complications arise in considering food. It has been shown (Howe, 1949*b*) that the larvae grow more quickly in food composed of fine particles than in one of coarse particles, but as the period of development is increased by less than 1 week this can have a maximum effect of 0.02 on r . Possibly more important is the effect on oviposition of the staling of food. Provision of new food in oviposition experiments always causes a stimulation of egg production, and in the experiments considered here it was not always possible to renew food regularly.

This shows up in Tables 1 and 2, fresh food being the cause of all the sudden increases of weekly egg number recorded. Depression of oviposition due to staling of food shows in a very short period. Howe (1951) found the egg laying per day in a trap food left in a warehouse for 2 days was much higher than in a similar food left for 2 weeks. It is not obvious if this decrease in oviposition is due to removal of food values, to addition of excreta or to some other cause, but it occurs very easily and quickly and may have a significant effect on the increase of warehouse populations. It is, of course, possible that an increasing proportion of eggs are eaten as they become more numerous, and this may also happen in tubes.

(d) Light

It is well known (Bentley, 1944) that Ptinid beetles are considerably more active in darkness than in light. Howe (1951), in an experiment simulating warehouse conditions, found that the daily egg number was markedly increased when it was necessary to keep an experimental bin in darkness continuously for a day instead of exposing it to the normal diurnal light changes. Most of the heavy infestations of Ptinids noted by this Laboratory have been in dark premises.

(e) Density and disturbance

Crowded conditions retard the rates of development and of oviposition and increase mortality. Mechanical disturbance of infested produce has similar effects, and both can prevent a species increasing at a maximum rate. Ptinids are subject to a crowding effect at low density (Gunn & Knight, 1945) which increases the period of development by about a week, although there remains ample food and space for the growing larvae. Presumably some such factor operates in warehouses more or less continuously and prevents r reaching its maximum. The values of r for *P. tectus* obtained at 25° C. using oviposition figures for isolated pairs and groups of adults are 0.38 and 0.33 respectively.

(f) Disease

Mortality figures used in this work were based on disease-free laboratory stock. In warehouses, a higher mortality is probable as a result of predators, parasites, and diseases. Cultures of *Niptus* and *Trigonogenius* have been seriously affected by a bacterial wilt disease, and D. W. Hall (unpublished) has found gregarines and nematodes in *Tipnus unicolor* (Pill. & Mitt.). In Germany *Niptus* is heavily affected by protozoal diseases (Eichler, 1939).

MODIFICATIONS MADE NECESSARY BY VARIABLE DEVELOPMENT

In the calculations made so far, it has been considered sufficiently accurate to use the mean period of development of all individuals regardless of sex to determine when oviposition commences. This is valid for most of the Ptinids because the

coefficient of variation is small, generally about 10 %, but for species such as *Ptinus sexpunctatus* for which the coefficient of variation is of the order of 30–50 %, this simplification must lead to inaccuracy. Therefore, for this species the value of r has been recalculated using the results for the development period of individual females instead of the mean for all individuals of both sexes. Since the egg-laying performance of these individuals is not known each is assumed to show the mean performance of the species. In the original calculation egg laying was reckoned as commencing in week 27 and continuing to week 54. In the recalculation the first adult begins to lay in week 13, nearly half have started by week 27 and the last starts in week 33, finishing in week 60. The effect of this is to spread the egg laying over a longer period, and in particular to introduce it into the period between 13 and 27 weeks. This change raises the calculated value of r from 0.044 to 0.054 (Table 3). In this experiment females developed more quickly than males but the difference was not statistically significant.

THE SIZE OF r IN WAREHOUSE CONDITIONS

It is theoretically possible to determine λ , the finite rate of increase in the warehouse, but in practice the making of an estimate is hampered by the difficulty of measuring the population. Howe (1950) gives figures for the populations of adult and immature *P. tectus* in a few small experimental bags of wheatfeed in a warehouse. The population growth was slow for the first 15 weeks during January to May. Over the next 8 weeks the weekly rate of increase of adults and immature stages inside the bags was 27 % and of adults found on the outside of the bags was 14 %. In 13 weeks from July to October, however, the weekly rate of increase was little over 1 % inside the bags and only 6 % for adults on the outsides of the bags. This suggests that the density of Ptinids in the bags, about 800 per kg., is near the maximum density permitting increase of population. Since Gunn & Knight (1945) found only about a 24 % retardation in the speed of development at a density of 6000 young per kg., this suggests that oviposition is depressed or eggs eaten at the higher densities. An increase of 27 % per week, i.e. $\lambda = 1.27$, is equivalent to an r value of 0.24. A retardation of 24 % in development speed would by itself reduce λ to 1.20.

It is difficult to predict warehouse values of r from laboratory results. With environmental conditions varying irregularly the age distribution may seldom be stable so that r values will not exactly describe the rate of increase. Nevertheless, it is worth examining the warehouse population.

To investigate egg output, a group of adult *P. tectus* were collected from a warehouse in February 1948 and divided into eight groups of fifty which were kept on wheatfeed in an unheated building. One batch comprising four of these groups was given drinking water three times weekly and continued to produce eggs for 70 weeks. The other batch was given one good drink at the beginning of the experiment and was then denied water. This batch laid eggs for only 10 weeks

(Table 5). Assuming a development mortality of 20 % (see Table 3) and a development period of 21 weeks ($2\frac{1}{2}$ cycles per year in British warehouses), r values of 0.151 and 0.124 can be calculated for the two batches. The high value obtained for the beetles denied water is entirely due to the high initial oviposition stimulated by the one drink given, again emphasizing the importance of the occasional drink in the

TABLE 5. *Egg number per female Ptinus tectus on wheatfeed in an unheated building over a period commencing in February*

Week	With drinks	Without drinks	Weeks	With drinks
1	1.7	1.9	11-15	10.3
2	4.7	4.3	16-20	15.3
3	3.6	2.0	21-25	14.5
4	5.2	3.3	26-30	11.9
5	5.6	4.4	31-40	8.3
6	2.9	1.6	41-50	3.2
7	1.3	0.5	51-60	1.7
8	3.3	0.2	61-72	1.6
9	4.3	0.1	—	—
10	4.7	0.1	—	—
Total	37.3	18.4	Total	104.2

TABLE 6. *Growth of population of Ptinus tectus associated with experimental bags in warehouse*

Period in store (weeks)	Adults on outside of bags (no.)	Percentage increase per week	All stages inside bags (no.)	Percentage increase per week
15	800	—	9,250	—
(15-23)	—	14.1	—	27.1
23	2,300	—	62,900	—
(23-36)	—	6.2	—	1.3
36	5,000	—	74,250	—
(15-36)	—	9.1	—	10.4

warehouse. The rates of increase are equivalent respectively to fifty and twenty-five times in 6 months, and show the possible size of r in the spring in England. Another batch of 200 beetles collected in the warehouse were kept in groups of 50 at 25° C. The longest lived survived 53 weeks, 3 weeks longer than the longest lived of the laboratory stock at 25° C. The average total egg number per female was about 220, about fifty below that of the laboratory stock, but laying was more concentrated into the first 6 weeks than with the laboratory stock. This, presumably, was due to the stimulation of high temperature and drinking water and may be a sign that oviposition in the warehouse is low. The sex ratio of the warehouse beetles was close to unity and their age distribution was presumed to be typical for natural population.

These experiments show that the oviposition of warehouse beetles is reasonably represented by the performance of laboratory populations given occasional drinks. This is important because estimation of the effective egg number per female in the

warehouse is very difficult. Howe (1951) measured daily variation in the egg output of a population of unknown size. He concluded that on average each female laid one egg a week in the warehouse during August. This figure is equivalent to an increase of 15 % per week at 20° C., an r of 0.1 per week. To obtain the value of r of 0.24 recorded for the experimental bags placed in this warehouse, seven eggs per female per week must be laid at 20° C.

The speed of development can be taken from laboratory results provided the relative humidity is above 50 %. Except under very dry conditions and limiting temperatures, developmental mortality in the laboratory remains at about 20 % and is assumed to be the same in the warehouse.

It is interesting to try to attempt to estimate r from warehouse temperatures using laboratory results including egg laying of groups of beetles given water to drink, i.e. the estimate is expected to be high. A stable age distribution throughout is assumed which involves a change from about 10 % of adults in winter to 5 % in summer, although in fact a higher proportion of adults may always be present. Also a straight-line relationship is assumed to exist between r and temperature because few points (see Table 3) are available to show what true relationship exists. Extension of this line to $r=0$ indicates that no increase is possible below 9.5° C. This is too high since some oviposition and slow development take place at lower temperatures. Using mean temperatures estimated from the graph of temperature maxima and minima given by Howe & Burges (1951) for 1948-9 it would appear that between October 1948 and April 1949, multiplication was possible during 1 week only, but that for the remainder of the year an average value of 0.19 per week for r could be achieved with a maximum of 0.31 in July.

These figures are in accord with those mentioned above as observed in the warehouse.

An unsuccessful attempt was made to show that *P. tectus* would overcome the other species in warehouse conditions. Two hundred adults of each of *P. tectus*, *Trigonogenius*, *Stethomezium*, *Gibbium* and *Eurostus* were placed in each of two dustbins of wheatfeed in June. One population was given water once a week. The population denied water was examined for adults after 7 months (January); all the *Eurostus* found were dead and only twenty-five live *Gibbium* were discovered. The remaining species each totalled nearly 400. A final examination was made 8 months later (September). Only two *Trigonogenius*, three *Stethomezium* and fifteen *Ptinus tectus* adults were found alive. No *Eurostus* or *Gibbium* were found, and only a few dead adults of the other species, except *Ptinus tectus* which exceeded 300. These results agree with expectation. In the other bin, when first examined after 12 months (June) all *Eurostus* found were again dead, but there were seventy-two live *Gibbium*. The other species had all multiplied, but less than 100 *Ptinus tectus*, 200 *Trigonogenius* and 400 *Stethomezium* adults were alive, together with 750 dead *Ptinus tectus* and 300 of each of the other two species. Four months later (October), there were 300 live *Trigonogenius* but fewer than twenty *Ptinus tectus* and *Stethomezium*; only

Ptinus tectus was present dead in appreciable numbers, viz. 130. In this bin, the insects given drinking water failed to multiply as well as expected. *P. tectus* obviously began to increase but failed during the winter and spring, so that *Trigonogenius* was able to become dominant. As expected *Eurostus* was unable to compete and *Gibbium*, a tropical species, did not increase. *Stethomezium* showed that it was able to increase during a British summer.

This paper forms part of the programme of work of the Pest Infestation Laboratory and is published with the permission of the Department of Scientific & Industrial Research.

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THE RAPID DETERMINATION OF THE INTRINSIC RATE OF INCREASE OF AN INSECT POPULATION

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(With 6 Text-figures)

The primitive (single oviposition period) method for determining the finite rate of natural increase (λ) of an insect species which lays all its eggs quickly is described, together with a summary of a more accurate method introduced by P. H. Leslie and L. C. Birch of calculating the infinite (infinitesimal) rate of increase (r) of any species. The parameter r is the natural logarithm of the parameter λ . Two methods of simplifying this more accurate method are discussed and illustrated by six examples which are used to compare the results given by all four methods.

The first modification of the Leslie-Birch method provides a means of representing a long oviposition cycle by a single figure so that the final calculation resembles the primitive single period method. The oviposition period is divided into a number of convenient unit periods, and using a table of weighting factors provided, the number of eggs laid in each of these unit periods is converted into the number of eggs required to be laid in the first of these unit periods of oviposition to make an equivalent contribution to the rate of increase. The equivalent oviposition figures for all the unit periods are summed to provide one figure which represents the observed egg number and pattern.

The second modification provides a means of representing the observed egg pattern by a constant rate of oviposition. This requires the same table of weighting factors but also needs a series of charts which are provided. This method usually gives a good answer at the first attempt, whereas the first modification usually requires two trial and error solutions to give an accurate estimate of r and hence of λ . Two of the examples show how the methods may be used for species for which information is scattered in the published literature. The minimum requirements for estimation of r are information on the length of the developmental cycle, the rate of egg output of adults, mortality of all stages and the sex ratio.

I. INTRODUCTION

In the study of the biology of insect pests of economic importance, one of the ultimate objects is, generally, to make an estimate of the rate at which the pest population will grow. Thompson (1931) developed a mathematical method for considering a situation in which a number of adults of an insect enter a new environment and begin to lay eggs. For a long-lived species with overlapping generations this method entails the use of some complex formulae and appears to be rather laborious. While Thompson's formulae are appropriate in certain individual instances, it is more usual to use a simpler and more approximate method to convert the results of a biological study into a general statement about the rate

at which a species can increase in numbers. The calculations involved are simple, but the method can be used only if the period in which eggs are laid is short compared with the time required for development, because it is necessary to assume all eggs are laid at a single instant, usually the mid-point of the oviposition period. Thus, if the developmental period of the species is d units, and the oviposition period is l units, all the eggs of the next generation are assumed to be laid after $d + \frac{1}{2}l$ units. If the female lays $2n$ eggs of which a proportion p mature, and the sex ratio of offspring is unity, then she produces np mature female offspring. Hence it is assumed that each female produces np female offspring in $d + \frac{1}{2}l$ periods so that the self-multiplicative rate of the species, which we may call λ , is calculated as the $d + \frac{1}{2}l$ th root of np . Taking an example: a species develops in $5\frac{1}{2}$ weeks and lays all its eggs in a week. Hence all the eggs are assumed laid at week 6. If the mean egg output per female is 60, the developmental mortality is 20% and the sex ratio of the survivors is unity, then the mean number of mature female offspring per female is $30 \times 0.8 = 24$ and the self-multiplicative rate of increase, λ , is $\sqrt[6]{24} = 1.698$. This simple calculation gives results which apply when generations keep in step with adults occurring together, but it also holds when the age distribution of the pest population is fixed and all stages of the insect are increasing at the same rate. Such a state is very rare in practice but it represents the situation to which growing populations tend and as such is useful in deciding whether the population will increase to epidemic proportions. Also it is obvious that slightly different results will be obtained if the eggs are assumed to be laid at some point other than the mid-point of the oviposition period, the highest result if the beginning of the period is used and the lowest result if the end is used. There is no logical general rule to show which is the best point to use; the mid-point seems justified by simplicity, although it normally gives an answer slightly below the true rate of increase. The discrepancy is small if the oviposition period is short but increases as this period lengthens (see Fig. 1), so that it cannot be used with confidence if the oviposition period exceeds about 4 weeks. Thus while for *Lasioderma* which lays eggs for less than 2 weeks the value of λ calculated in this simple manner is 1.602 per week compared with 1.616 per week obtained later in this paper by more accurate methods, the discrepancy for *Tribolium* which lays eggs for 25 weeks is much greater. The value of λ for this species obtained by Leslie & Park (1949) is 2.028 per week against 1.456 from the simple method placing all the eggs laid at the middle of the oviposition period. Fig. 2 shows graphically the population growth indicated by a number of values of λ .

II. THE METHOD OF LESLIE AND BIRCH

In recent years, Leslie (Leslie & Ranson, 1940) has modified methods used in human demography and made them suitable for application in zoology. A parameter which is denoted by the letter r and is called the intrinsic (infinitesimal) rate of increase is calculated by methods given in detail by Birch (1948) and by Leslie &

Park (1949). This parameter r is the natural logarithm of the self-multiplicative rate of increase λ mentioned above, and like that parameter applies only to a population with a stable age distribution. This parameter may be calculated for any kind of life cycle. If the oviposition period is long it may be divided into a number

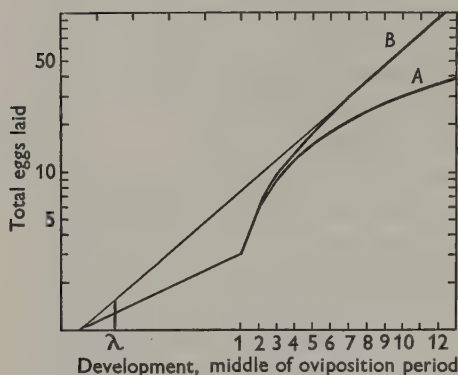


Fig. 1.

Fig. 1. Diagram showing the size of error caused by using the simple method of calculating λ . In the example graphed, the developmental period (d) is 4.5 units, and 3 eggs per period are laid. The lower curve (A) plots on a logarithmic scale the cumulative sum of eggs laid during the number of periods (l) shown, the eggs being placed at the middle point of the oviposition period ($d + \frac{1}{2}l$). The upper curve (B) gives the equivalent egg number calculated by a more accurate method. For any length of oviposition period, the values of λ may be estimated by joining the appropriate point on each curve to the origin by a straight line which cuts $x = 1$ at λ , as shown for $l = 1$. The maximum value of λ for this oviposition rate is shown.

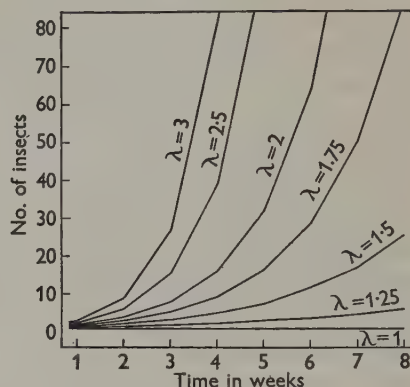


Fig. 2.

Fig. 2. Curves for various values of λ per week, showing population growth represented by each value.

of convenient units. The main disadvantage of the Leslie-Birch method is that although the mathematical operations are not difficult, they involve the use of trial and error solutions of an equation, each involving the summation of values obtained from exponential tables, a procedure which may be tedious and sometimes lengthy.

This paper attempts to simplify this method introduced by Leslie and by Birch for use by biologists who are not mathematically inclined, so the method is first summarized. The basic requirements are the female life table and an age-specific fecundity table. The life table gives the probability at birth (l_x) of a female being alive at any stated age x , and the age-specific fecundity table gives the mean number of female offspring (m_x) produced per unit of time by a female of age x . These data are normally available in any complete study of an insect species. A life table can be constructed by following the life history of a group of insects through from their birth (egg laying) to emergence as adults, and recording all deaths as they occur together with the sex of those which die as adults. Recording

the eggs as they are produced by the adults gives the data for an age-specific fecundity table. It is often more convenient in experiments to deal separately with the developmental and adult parts of the tables. Then a figure for a mean developmental period is obtained from one experiment and the age-specific fecundity table obtained using freshly emerged adults, the age of the females at the beginning of this experiment being taken as the mean developmental period. This is the form of results used by Birch (1948) and by Leslie & Park (1949), and is the form normally required by the simplifications made in this paper.

The developmental cycle and adult life is divided into convenient units, say weeks, which are tabulated in the first column of a working table. Egg laying and death are assumed to occur at the mid-point in each week and these results are also included in the table. Thus if development takes 4 weeks and the adult lays eggs in its first week of life then these eggs are assumed laid at 4.5 weeks and form the first entry of the m_x column as in the table below. Adult mortality in the first week plus the whole developmental mortality is calculated as a fraction, and its complement is placed against 4.5 weeks as a survival rate in the l_x column. Another column gives the product of egg per live female per week (m_x) and survival rate (l_x), which product is designated k_x in this paper, viz.

Age group	Pivotal age (x)	(m_x)	(l_x)	(k_x)
4-5	4.5	20.0	0.87	17.400
5-6	5.5	23.0	0.83	19.090

The table is continued until oviposition ceases or until k_x is small compared with the early entries in the table. The next stage of the calculation is conveniently put into another table in which the first two columns show the pivotal ages x , and the values of k_x . A preliminary value of r is now estimated by the method explained later, on p. 141, and a column for the value $-rx$ inserted in the table. Now from the tables of the exponential function (e.g. Comrie, 1950) values of e^{-rx} are inserted as the next column of the table. Finally, each k_x is multiplied by its respective value of e^{-rx} and all the products $k_x e^{-rx}$ are summed. Various values of r are tried until the sum is brought as close as possible to unity, since, if the exact value of r is obtained the summation would total precisely 1. The relationship between r and λ is shown graphically in Fig. 3.

III. SIMPLIFICATIONS

Since the requirements of trial and error solutions using exponential tables and extensive additions are likely to deter some biologists from using this very valuable parameter, it is worth while exploring the possibilities of simplifying or shortening the procedure without undue loss of accuracy. One attractive idea is to try to represent the observed egg pattern by a number of eggs laid at a single instant and then to use the simple form of calculation mentioned in the Introduction of

this paper. Another idea is to represent the egg pattern by a constant rate of oviposition over a period similar to, or longer than, that observed. Both ideas can be realized by a manipulation of the basic equation of the Leslie-Birch method. Both require tables of weighting coefficients which are given in Table 1. These are calculated from and replace the tables of the exponential function used in the original method. It can be seen readily from Table 1 and from Fig. 4, which illustrates Table 1 C graphically, that as the value of r increases the weight given to later periods of oviposition diminishes rapidly. Hence a trial and error solution

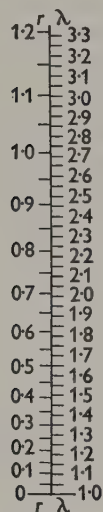


Fig. 3.

Fig. 3. Diagram showing the relationship of the numerical values of r and λ .

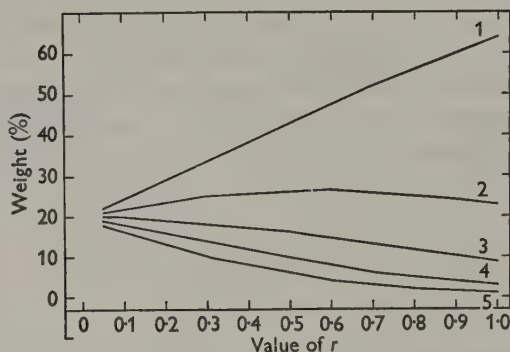


Fig. 4.

Fig. 4. Proportional weight ascribable to the oviposition of various periods for various values of r when egg laying occupies 5 periods (see Table 1 C).

is still required, because r is calculated from figures for oviposition which must themselves be weighted according to the value of r . The actual procedure, however, is quite simple as is shown by the examples given below.

If the second idea is used, a series of charts (see Fig. 6) are needed in addition to Table 1. Using these, an extremely good estimate of r is likely to be obtained at the first attempt. The previous procedure is simpler but, in general, requires more than one trial if it is to give a good estimate of r .

The five different weighting tables of Table 1 and the five charts of Fig. 6 could be replaced by a single table and a single chart covering a long oviposition period, say 20 weeks, but there seems no advantage in doing this. If such a table were presented with percentage weights expressed to the nearest integer there would be

undue loss of accuracy for short oviposition periods, while if the weights were given, say, to two decimal places, the calculations would be no simpler for long oviposition periods than if the original exponential tables were used.

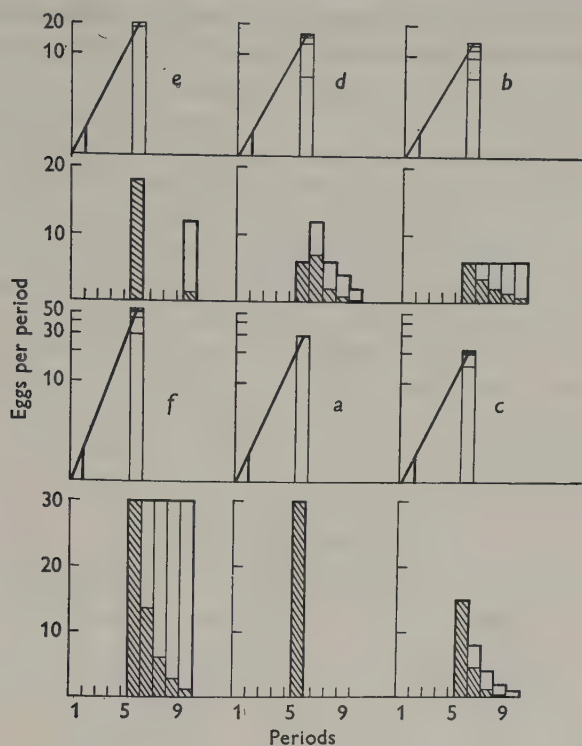


Fig. 5. Diagrams showing the effect of various types of egg pattern on r . The lower figure in each pair represents the actual oviposition on an arithmetic scale, the shaded areas representing the egg number required in the first week of laying to make an equivalent contribution to r . In the top figure of each pair the shaded areas are all transferred to the first week of laying and plotted on a logarithmic scale. The value of λ ($\text{antilog}_e r$) is shown for each.

A. Representation of egg pattern by a single figure

Both modifications of the Leslie-Birch method will be illustrated here by the examples already published in the papers describing the method, one with *Calandra oryzae* L. (Birch, 1948) breeding at 29° C., and the other with *Tribolium confusum* Duv. at 28° C. (Leslie & Park, 1949). Two examples are also worked out for the slower-breeding Ptinid beetles bred at 25° C. from Howe (1953), and the results compared with those obtained by the original mode of calculation. Finally, for the two species *Lasioderma serricorne* (F.) and *Tenebrio molitor* L., the rate of increase is calculated from information scattered in the literature.

Before these examples are discussed a further simplification of procedure can be mentioned. In constructing the age-specific fecundity table for man, the birth-rate is normally expressed in terms of females alive at the time of the births. In

TABLE I. *Proportional weights (expressed as percentage) attributable to the egg output of various periods in order to calculate the weighted mean egg number per period*

A. Oviposition in 2 periods												
<i>r</i>	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1
Period 1	51	52.5	55	57.5	60	62	64.5	67	69	71	73	75
„ 2	49	47.5	45	42.5	40	38	35.5	33	31	29	27	25
B. Oviposition in 3 periods												
<i>r</i>	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1
Period 1	35	37	40	44	47	50	54	57	61	64	66.5	69
„ 2	33	33	33	32	32	31	30	29	27	26	24.5	23
„ 3	32	30	27	24	21	19	16	14	12	10	9	8
C. Oviposition in 5 periods												
<i>r</i>	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1
Period 1	22	24	29	33	38	43	47	52	56	60	64	67
„ 2	21	22	23	25	25	26	26	26	25	24	23	22
„ 3	20	20	19	18	17	15	14	13	12	10	9	7.5
„ 4	19	18	16	14	12	10	8	6	5	4	3	2.5
„ 5	18	16	13	10	8	6	5	3	2	2	1	1
D. Oviposition in 7 periods												
<i>r</i>	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1
Period 1	17	19	24	30	35	41	46	51	55	59	63	67
„ 2	16	17	20	22	24	25	25	25	25	24	23	22
„ 3	15	15	16	16	16	15	14	13	11	10	9	8
„ 4	14	14	13	12	11	9	8	6	5	4	3	2
„ 5	14	13	11	9	7	6	4	3	} 4	3	2	1
„ 6	13	11	9	7	5	3	2	} 2				
„ 7	12	10	7	5	3	2	1					
E. Oviposition in 11 periods												
<i>r</i>	0.5	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1
Period 1	12	14	20	27	33	40	45	50	56	59	63	67
„ 2	11	13	17	20	22	24	25	25	25	24	23	22
„ 3	10	12	14	15	15	16	14	12	11	10	9	7
„ 4	10	11	11	11	10	9	7	6	} 7	6	4	3
„ 5	9	10	9	8	7	5	4	3				
„ 6	9	9	8	6	5	3	2	} 3	1	1	1	1
„ 7	9	8	6	5	3	1	1					
„ 8	8	7	5	3	2	1	1	} 1	0	0	0	0
„ 9	8	6	4	2	1							
„ 10	7	6	3	2	1	} 1	1	1	0	0	0	0
„ 11	7	5	3	1	1							

experiments with insects, however, the oviposition rate can as easily be expressed per female alive at the *beginning* of an experiment,* which eliminates one step from the calculations, and also reduces the life table required for the calculation

* This is only valid if there are no losses, and should not be used if more than 5% of females are lost during an experiment.

of r to a single figure giving developmental mortality. If the stable age distribution is required, then details of the mortality rate must be available.

Birch (1948) gave the weekly oviposition rate of *Calandra oryzae* (per female alive at time of laying) for the first 5 weeks as 40, 46, 30, 25 and 25. Correcting these figures for sex ratio and mortality he obtained the figures 17.4, 19.1, 12.2, 10.0 and 9.9 in the k_x column of his working table. Birch found that the first 3 weeks of oviposition accounted for 94% of the value of the rate of increase, and the first 5 weeks accounted for 99%, so it is not necessary to consider any later oviposition. This is typical; in practice only the early part of the oviposition is important in rapidly breeding insects but a longer oviposition period must be considered when the insect breeds more slowly. Since the developmental period (d) is 4 weeks, if eggs were laid only during the first week of adult life, then by the primitive calculation given in the Introduction, the self-multiplicative rate of increase λ would be $\sqrt[4]{17.4}$, and $\log \lambda = \log 17.4/4.5$. Since $r = \log_e \lambda$, therefore $r = (\log_e 17.4)/4.5$ or approximately 0.6. The 19.1 eggs laid in the second week of adult life contribute less to the intrinsic rate of increase than a similar number laid a week earlier, and therefore their effect is represented by a smaller number of eggs laid in the previous week. The table of relative weighting coefficients gives the factors by which each week's egg number must be multiplied to transfer its effect to some other week, conveniently the first. Now the true value of r must, in fact, be greater than 0.6 so a value of 0.7 may be taken as a first trial value. Using this trial value, we will calculate r using Table 1 B giving values for oviposition in three periods. For a 3-week oviposition period the weighting coefficients for each week for $r=0.7$ are 57, 29 and 14.

The equivalent egg number in the first week can be calculated as

$$17.4 + \frac{2.9}{5.7} \times 19.1 + \frac{1.4}{5.7} \times 12.2 \quad \text{or} \quad (17.4 \times 57 + 19.1 \times 29 + 12.2 \times 14)/57 = 30.1.$$

Similarly, the equivalent egg number in the first week for the first five weeks of oviposition is

$$(17.4 \times 52 + 19.1 \times 26 + 12.2 \times 13 + 10.0 \times 6 + 9.9 \times 3)/52 = 31.7$$

and in the same way for 11 weeks it is 32.3. Thus $4.5r = \log_e 30.1$, or alternatively $\log_e 31.7$ or $\log_e 32.3$ according to the length of oviposition period considered. These give values of 0.757, 0.768 and 0.772 per week respectively. The value calculated by Birch is $r=0.762$ per week, the discrepancy in the results obtained here being due to the use of weights appropriate to an r of 0.7. If an r value based on the 5-week period of oviposition is now recalculated using weights for $r=0.768$, obtained from Table 1 by interpolation, a new equivalent egg number is obtained as follows:

$$(17.4 \times 54.7 + 19.1 \times 25.3 + 12.2 \times 12.3 + 10.0 \times 5.3 + 9.9 \times 2.3)/54.7 = 30.4,$$

whence $r=0.759$ per week. If these weighting values are rounded to the nearest integer, the equivalent egg number becomes 30.0, and r is affected in the third decimal place. Thus it is evident that if the estimate of r is required to be accurate

to three places then the weighting figures given in Table 1 should be improved to give the first decimal place.

The results of Leslie & Park (1949) can be treated similarly. Their fecundity table gives the mean number of daughter eggs deposited per 11 days per female alive in each age group and they give the developmental period (d) of *Tribolium castaneum* Herbst. to the adult stage as 33 days. We will therefore use 11-day periods as units. The developmental period (to the middle of the first period of laying = $d + \frac{1}{2}l$) as defined in this paper is 3.5 periods and the effective female egg output per female (k_x) given by Leslie & Park for the first seven periods are 28.2, 41.4, 42.4, 39.1, 28.5, 24.0 and 21.9. The remaining nine periods are disregarded as unimportant because r is evidently high. The minimum value for r is obtained from the equation

$$3.5r = \log_e 28.2 = 3.34, \quad \text{or} \quad r = \text{about } 1.$$

Using the weighting values for $r = 1$ from Table 1 D, the equivalent egg number in the first week of adult life is found in the usual way from

$$[28.2 \times 63 + 41.4 \times 23 + 42.4 \times 9 + 39.1 \times 3 + (28.5 + 24.0 + 21.9) \times \frac{2}{3}] / 63$$

to be 52 and r from the equation $3.5r = \log_e 52$ is therefore 1.129 per 11-day period. If the procedure is now repeated using the weights for $r = 1.1$ the equivalent egg number is recalculated as 48.39, whence $r = 1.08$ per 11 days or, dividing by 11, 0.101 per day, or 0.705 per week. Leslie & Park obtained a value of 0.707 per week.

Two further examples with lower values of r are taken from Howe (1953) on *Trigonogenius globulus* Sol. and *Ptinus fur* (L.) at 25° C. *Trigonogenius* has a developmental period ($d + \frac{1}{2}l$) of 11 weeks to the middle of the first week of laying and lays eggs for 50 weeks. Although 4 to 5 eggs per female per week are still being laid after 25 weeks we will ignore all but the first 11 weeks of oviposition. The weekly egg numbers (per female alive when oviposition starts) to be considered are 14.2, 7.9, 10.7, 8.8, 7.0, 6.9, 3.4, 4.6, 4.7, 2.8, 3.1; these are multiplied by 0.3 to adjust for pre-adult mortality (40%) and sex (1:1). Since r is expected to be low, the weight to be given to each week of oviposition decreases slowly, the original guess of the equivalent egg number is taken as the total of the first 5 weeks, namely 16. Therefore $11r = \log_e 16 = 2.77$ and $r = 0.25$. Weights for this value of r are used and the new equivalent egg number obtained is 10.61 which gives $r = 0.215$ per week. Further repetition gives the same value, 0.227, as found by the full method.

Ptinus fur has a developmental period of 21 weeks to the middle of the first week of laying and lays all its eggs in less than 20 weeks. The maximum egg number per week is 6.3, and all egg numbers must be multiplied by 0.28 ($\frac{1}{2}l_x$) to allow for pre-adult mortality and sex. Unit periods of a fortnight are used and the oviposition results used up to week 14 (seven periods), only 0.1 egg per female being laid after that. Uncorrected egg numbers per fortnight ($2m_x$) are 2.4, 10.1, 11.0, 7.3, 6.2, 1.4, 0.2. Again r is obviously low so the equivalent egg number is taken as the

sum of the first three laying periods, namely about 7. Since the developmental period ($d + \frac{1}{2}l$) is 10.75 periods and $\log_e 7$ is approximately 1.9 the first guess for r is about 0.2. Using the weights given in Table 1D the new estimate of the equivalent egg number is 7.15 and r is 0.183 per fortnight; a final value is 0.186. The value obtained by the full method was 0.094 per week which is equivalent to 0.188 per fortnight.

Since all the four examples used give good results when compared with the orthodox full method we can now turn to species for which r has not been calculated but for which some basic information is available in the literature. Dick (1937) gives daily egg-laying rates for *Lasioderma serricorne* (F.) at 27° C. Fraenkel & Blewett (1943) give the developmental period of this species on wholemeal flour up to the appearance of the adult in the cocoon as about 5 weeks at 25° C., 70% R.H., with a mortality of 10%. Runner (1919) gives the period spent in the cocoon as nearly a week, and Dick gives the pre-oviposition period at 27° C. as nearly a week. Thus we can take the developmental period to the middle of the first week of oviposition as 7 weeks and the sex ratio as unity (Runner, 1919). The observed weekly egg numbers per female ($2m_x$), 42.5 and 33.6 (Dick), are corrected by the multiplication factor ($\frac{1}{2}l_x$) 0.45, to 19.1 and 15.1. Since r is likely to be quite high and the second week of laying likely to carry only about half the weight of the first we will take the equivalent egg number as 25. Thus $7r = \log_e 25 = 3.2$ and r is about 0.45. Using the weighting coefficients 61 and 39 obtained by interpolation from Table 1A, the new estimate of the equivalent egg number is 28.75 and the new estimate of r is approximately 0.480 per week, in the 25–27° C. temperature zone.

Of the other species for which Dick (1937) examined the oviposition performance, *Stegobium paniceum* (L.) resembles *Lasioderma*, and *Tribolium confusum* Duv. resembles *T. castaneum*, but *Tenebrio molitor* exhibits a special difficulty also encountered with *Ptinus sexpunctatus* Panz. (Howe, 1953). These species both have a very variable rate of larval development, so that eggs laid at the same time may become mature fertile adults at widely separated times. Therefore the assumption made in all previous examples, that all eggs laid become mature at a later date fixed by the mean developmental period is not valid. This difficulty can only be overcome by treating independently the development and oviposition of each female. Here an approximation is made by taking the spread of development of a sample as typical for the species as a whole and applying the mean oviposition curve given by Dick to groups of females having similar periods of development, so forming a new composite oviposition curve for the species. Dick (1937) gives the oviposition rate of *Tenebrio* per 8-day period and also gives 8 days as the length of the pre-oviposition period at 27° C. Leclercq (1950) gives graphically the larval developmental period at 27° C. His results have been regraphed on probability graph-paper in order to determine when one-fortieth, three-fortieths, and so on to thirty-nine-fortieths of the larvae had pupated. These values, given in Table 2, are

taken as the mid-points of the developmental periods of twenty successive groups of larvae. To these values are added 7 days for the egg period and 8 days for the pupa (see Table 2). The developmental period of the quickest developing group of larvae is calculated in this way to be 123 days so that oviposition is assumed to commence 8 days later, on day 131.

The slowest growing individuals are similarly estimated as beginning to lay on day 245 and to cease laying after 337 days. For periods of development and oviposition of this length, 14-day units are convenient. Oviposition starts during the 14-day period centred on 126 days so the length of development to the middle of the first laying period is 9 fortnights. Using Dick's figures for mean oviposition per 8 days, the theoretical oviposition rate for each of the 14-day periods centred successively on day 126, 140, 154 and so on, can be tabulated for each of the twenty groups into which Leclercq's larvae are divided (Table 2) and so the oviposition per period of the whole group calculated. This is found to extend over sixteen periods, in the last four of which the oviposition rate is below one-half an egg per period. The calculated rates of oviposition are given in Table 2. Developmental mortality is assumed to be 10% and the sex ratio unity so that a correction factor ($\frac{1}{2}l_x$) of 0.45 is used to convert the oviposition figures ($2m_x$) to effective female eggs per female (k_x). The first eleven oviposition periods only are considered. A fairly low value is to be expected for r so the equivalent egg number is taken as the sum of the first five periods, which is approximately 14. A tentative value for r is obtained from the equation $9r = \log_e 14 = 2.6$, whence $r = 0.3$ per fortnight. Now the weights given in Table 1E are used to obtain a new estimate of the equivalent egg number which is found to be 8.7, r being 0.240 per fortnight. Further adjustments give 0.270, 0.255 and a final value of 0.260.

The disadvantage of this modification is the probability that several trial and error repetitions will have to be made because there is no sound guide to the value to use as a first guess. It has, however, a useful safeguard that if a very low first guess is made, then the weighting factor used as a divisor will also be low and the first estimate of r will be too high, but will be closer to the true value of r . If the procedure suggested above is carried out each successive estimate of r will be more accurate, being in turn, too high and too low. This damped oscillation can be imitated to avoid the necessity for repeated calculation as follows: if an original guess for r is 0.4 and the corrected value obtained using the weights for 0.4 is found to be 0.8, successive means of pairs of values in a series beginning with the guess and the first estimate of r can be written down thus: 0.4, 0.8; 0.6; then 0.8, 0.6; 0.7; then 0.6, 0.7; 0.65, and so on giving in turn 0.675, 0.663, 0.669, 0.666, 0.668, 0.667. Any convenient value in this series could be used; 0.7 or 0.65 would probably give results of sufficient accuracy, but if the series is carried beyond 0.65, it would be as well to use the final value of 0.667 as the second estimate of r to be tried. This modification has the advantage that it resembles the primitive method mentioned in the Introduction. Also it is clear, as Fig. 5 shows, that each

TABLE 2. *Method of calculation of oviposition rate in eggs per female per fortnight of a Tenebrio molitor population using data on oviposition from Dick and on larval development from Leclerq*

(The very variable period of larval development is divided into twenty consecutive groups each representing an equal number of insects with a similar developmental period and for each group one-twentieth of the egg output per fortnight of a female is tabulated.)

Middle of oviposition period (days)	Length of developmental period: larval period (Leclerq) + 7 days (egg) + 8 days (pupa) + 8 days (preoviposition)																				Total
	131	146	154	160	165	170	174	178	182	186	189	193	197	201	205	210	215	221	230	245	
126	0.51	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.51
140	2.00	0.34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.34
154	0.87	2.10	1.36	0.34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.67
168	0.44	0.89	1.53	2.10	1.77	1.02	0.34	—	—	—	—	—	—	—	—	—	—	—	—	—	8.08
182	0.21	0.46	0.67	0.89	1.32	1.72	2.10	1.90	1.36	0.68	0.17	—	—	—	—	—	—	—	—	—	11.48
196	0.09	0.23	0.37	0.46	0.57	0.76	0.89	1.24	1.53	1.88	2.13	1.77	1.19	0.51	—	—	—	—	—	—	13.59
210	0.03	0.10	0.15	0.23	0.31	0.41	0.46	0.55	0.67	0.84	0.98	1.32	1.62	2.00	2.03	1.36	0.51	—	—	—	13.62
224	0.00	0.03	0.06	0.10	0.12	0.16	0.23	0.29	0.37	0.42	0.49	0.57	0.71	0.87	1.15	1.53	2.00	1.77	0.34	—	11.19
238	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7.64
252	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5.01
266	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.48
280	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.09
294	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.42
308	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.14
322	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.03
336	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.00

successive oviposition period adds to the size of r , but that each makes a relatively smaller contribution until eventually the increment added is negligible.

Fig. 5 (p. 139) illustrates diagrammatically how some egg patterns affect r . Five graphs are shown where the total female egg output per female is 30, one (a) where all oviposition occurs in one period and four (b) to (e) requiring five periods. These include (b) 6 eggs each period, (c) a peak of 15 in the first period followed by 8, 4, 2, and 1; (d) a smaller peak in the second period, i.e. 6, 12, 6, 4, 2; and (e) oviposition in two periods with a gap between, i.e. 18, 0, 0, 0, 12. Also included is (f) in which an even oviposition rate of 30 per period is maintained for five periods. For all, development is assumed to require five periods. These graphs cover the three main patterns of oviposition of insects, that where all eggs are laid quickly, that where egg laying is continuous and prolonged and that where the eggs are laid in batches with gaps during which none are laid, and show the exceptional importance of the eggs laid early in life.

TABLE 3. Values of λ per week calculated by the accurate Leslie-Birch method, by the modifications of this method and by the simple single period method. The maximum value obtainable by the last method is also given

Species	Length of oviposition period considered in weeks	Leslie-Birch method	Modifications		Single period method
			I Algebraic	II Graphical	
<i>Calandra oryzae</i>	15	2.143	—	—	—
	5	—	2.136	2.160	1.917
	2	—	—	—	2.053
<i>Tribolium castaneum</i>	25	2.028	—	—	—
	11	—	2.024	2.014	1.456
	3	—	—	—	1.964
<i>Ptinus fur</i>	15	1.099	—	—	—
	14	—	1.098	1.097	1.090
	10	—	—	—	1.096
<i>Trigonogenius globulus</i>	50	1.255	—	—	—
	11	—	1.255	1.252	1.214
	6	—	—	—	1.232
<i>Lasioderma serricorne</i>	2	1.614	1.616	1.616	1.602
<i>Tenebrio molitor</i>	32	1.136	—	—	—
	22	—	1.139	1.136	1.137
	16	—	—	—	1.145

B. Representation of egg pattern by a uniform rate of oviposition

This modification follows more clearly from the basic equation than the previous one and is likely to provide an accurate value for r with only one trial estimate. Its use, however, requires in addition to Table 1, a series of charts, examples of which are given in Fig. 6. The charts need to cover four variables, estimates of three of these being used to make an estimate of the fourth. One variable, the

length of the oviposition period (measured in terms of a selected unit, i.e. days, weeks or months) is covered by having a different chart for each individual number of oviposition periods. Charts for 2, 3, 5, 7 and 11 units are given here (Fig. 6). A second variable, the time from the laying of an egg to the middle of the first period in which an egg of the next generation is laid, briefly called the developmental period, is shown as a series of sloping lines across each chart. Lines for developmental periods of 5, 6, 7, 8, 9 and 10 units are given on each chart together with appropriate additional lines. The third variable, a number representing the oviposition rate in a unit period on a logarithmic scale, forms the vertical axis of each chart; and values of r , the variable to be estimated, are shown along the horizontal axis. As in the previous modification, the observed oviposition rate is adjusted for sex ratio and mortality to give the number of female eggs reaching maturity. To represent the oviposition pattern of the insect by a constant rate, a weighted mean per period is found, using the proportional weights given in Table 1. As mentioned previously, the proportional weights given to successive periods diminish and do so more rapidly the higher the value of r . Since the weight given to each period of oviposition depends on r , a weighted mean must first of all be guessed from the early weeks of laying, and a provisional value of r found. This value of r is used for reading off weighting coefficients for finding a new r . If the original guess of the mean was good, this r should be satisfactory, but if the guess was poor the process may have to be repeated using new weights appropriate to the new r . The r value is read from the charts as corresponding to the point of intersection of the development period line and the oviposition rate per unit period.

This process is illustrated by the six examples used for the previous modification. The adjusted oviposition rates for the first 5 weeks of laying for *Calandra oryzae* at 29° C. have been given on p. 141 as 17.4, 19.1, 12.2, 10.0 and 9.9. Since r will be relatively high, the early weeks will largely outweigh the later ones, so the preliminary guess for the equivalent constant rate of oviposition is taken as 18 eggs per week. The development period to the middle of the week in which the first egg is laid is 4.5 weeks, so interpolating midway between the 4- and 5-week lines on the 5-week oviposition chart (Fig. 6c) for 18 eggs per week we find r is about 0.8. The relative weights given to the weeks (Table 1C) are therefore 56, 25, 12, 5 and 2. These weights are used to find an ordinary weighted mean, viz.

$$(17.4 \times 56) + (19.1 \times 25) + (12.2 \times 12) + (10.0 \times 5) + (9.9 \times 2) / 100 = 16.7.$$

Using Fig. 6c, as before, this is found to correspond to an r of about 0.77, which compares with a true value of 0.762. In this example there is some difficulty in interpolating between lines on the chart. For the *Tribolium* examples Fig. 6d and Table 1D are used. From the adjusted mean oviposition rate for seven 11-day periods given on p. 142 the equivalent constant rate is guessed to be 30 per period. The developmental period to the middle of the period in which the first egg is laid is 3.5 periods, so again interpolation is necessary, this time between the

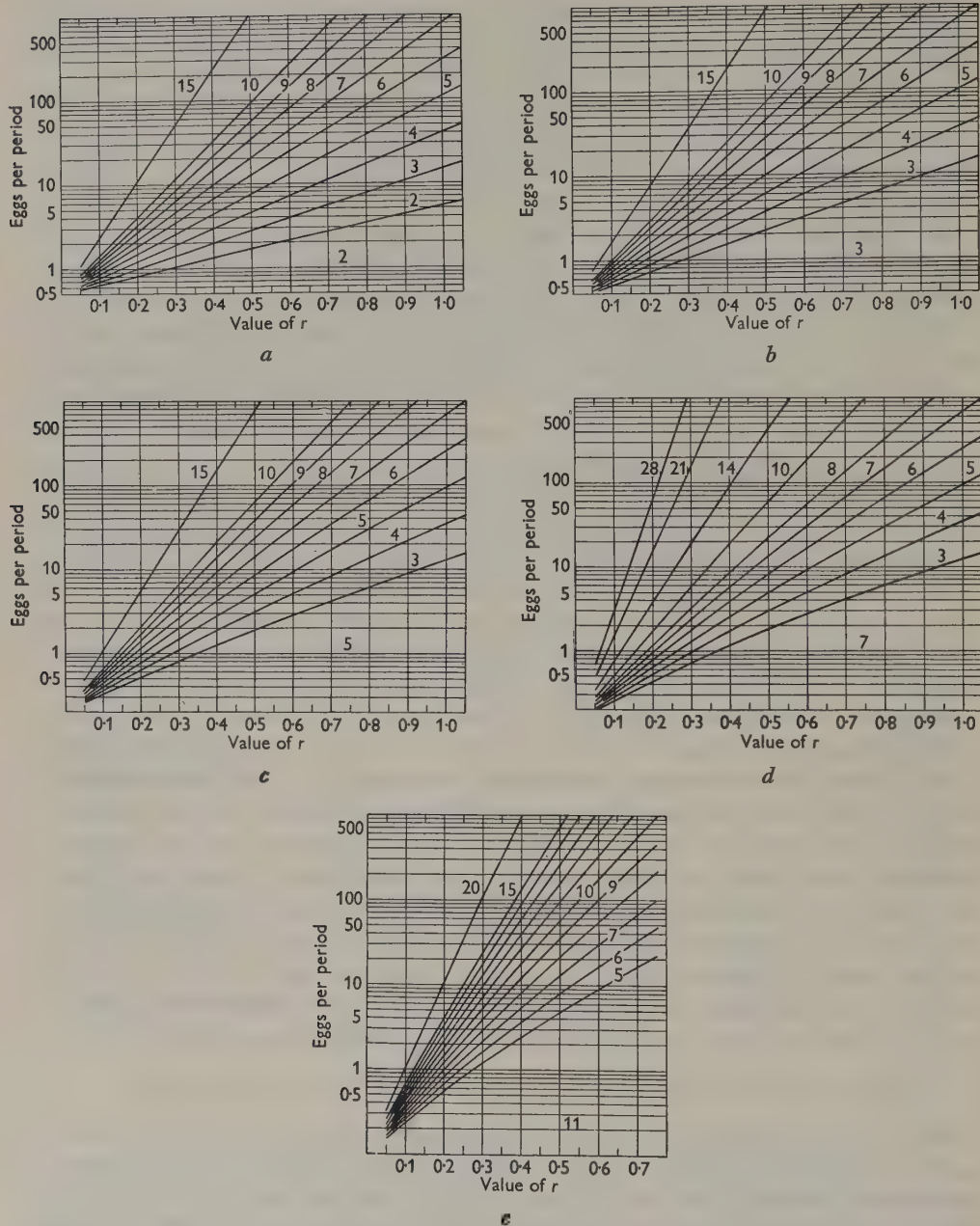


Fig. 6. Charts for use in calculation of r by method of representing observed egg pattern as a constant rate for the number of periods given in the bottom right-hand corner of each chart. The curves crossing the charts carry numbers showing the length of developmental period represented by each.

3- and 4-period development lines of Fig. 6*d*. The corresponding r value just exceeds 1.05, the highest value plotted in Fig. 6*d*, so the weighting coefficients for $r = 1.1$ from Table 1D are taken. The weighted mean constant oviposition rate obtained using these weights is 32.4 which again exceeds 1.05 and by extrapolation can be guessed as about 1.1 per 11 day period. The true value here was 0.707 per week and the value obtained on p. 142 from the previous modification was 0.705.

In the next example, which deals with *Ptinus fur*, interpolation is necessary between the 10- and 14-period lines in Fig. 6*d*, because the developmental period is 10.75 fortnights. For this species the constant rate of oviposition is guessed as being 2 per fortnight from the figures given on p. 142. From Fig. 6*d* the corresponding r is found to be about 0.2, and the weighted mean constant oviposition rate calculated from the weights given in Table 1D is 1.72. This in turn gives $r = 0.185$ per fortnight.

For *Trigonogenius* we use Fig. 6*e* and Table 1E, and figures for oviposition from p. 142. The preliminary guess of the constant oviposition rate is 2.5 and the corresponding value of r falls between 0.2 and 0.25. This gives a weighted mean of 2.59 eggs per week and a final r of 0.225 per week. For *Lasioderma* we use Fig. 6*a* and Table 1A. The adjusted constant rate of oviposition is 20 eggs per week whence r is about 0.5. Using the weighting coefficients 62 and 38, the weighted constant oviposition rate becomes 17.6 and r is about 0.48 per week. For *Tenebrio molitor*, we again use Fig. 6*e* and Table 1E, together with the oviposition results of Table 2, adjusted as on p. 144. The constant rate of oviposition is guessed as about 4 per fortnight so that from the 9-period development line r is found to be about 0.3. Using the weights given in Table 1E the weighted constant oviposition rate is 2.35 and r is therefore 0.255 per fortnight.

From the examples it is evident that these modifications of the Leslie-Birch method give results similar to each other and to the original method.

Conclusions

The methods described here enable a quick estimate to be made of the intrinsic rate of increase of species found in stored produce. These methods have the advantage of replacing by a simple trial and error method the more complicated calculation needed in the original method, but they do not give additional information such as the stable age distribution, which is easily obtained from the original method.

Table 1 and the charts given in Fig. 6 are considered sufficient for most situations, but if other combinations are needed they can easily be calculated from exponential tables and be tabulated or graphed. When less than eleven oviposition periods are needed but charts are not available, the charts given can be used by the device of adding periods with zero eggs at the end of the observed results.

IV. COMPARISON OF VALUES OF λ OBTAINED BY SIMPLE AND MORE ACCURATE METHODS

It is of interest to compare the values of λ obtained by the simple single-period method mentioned in the Introduction of this paper with those obtained by the other methods mentioned in this paper. The simple method is essentially the approximation $\sum_{k=1} e^{-rx}$ of the equation for calculating the intrinsic rate of growth applied to examples where all the egg laying is completed in a single period. The summation therefore contains only one term ke^{-rx} which may be written k/e^{rx} or, since $r = \log_e \lambda$, as k/λ^x , whence $k = \lambda^x$ or $\lambda = x\sqrt[k]{k}$. The symbol k represents the number of female eggs laid and growing into mature adults, r the intrinsic infinitesimal rate of natural increase, λ the finite rate of increase, and x the developmental period up to the middle of the single period in which oviposition occurred ($= d + \frac{1}{2}l$ of Introduction). Since $k = e^{rx}$ or $\log_e k = rx$, there is an obvious relationship between the egg number, the developmental period and the intrinsic rate of increase of species laying all their eggs in a short period.

The first modification of the Leslie-Birch method shows how long oviposition periods which must be subdivided can be treated, and Fig. 5 illustrates the procedure. It is clear that if a species with developmental period x lays k eggs in each of 2 weeks, the intrinsic rate of increase r is not the sum of the values r_1 and r_2 obtained by considering the 2 weeks separately, but is found from the equation $ke^{-rx} + ke^{-r(x+1)} = 1$. This is because the populations arising in the three situations have different age distributions. Thus taking a population in which $x = 6.5$ weeks, and $k = 20$ eggs, all mortality occurring at the end of the period of oviposition, then $6.5r = \log_e 20$, whence $r = 0.461$ and the stable age distribution contains 2.4% adults. If $x = 7.5$ weeks and $k = 20$ eggs, then $r = 0.399$ and the stable age distribution contains only 2.1% of adults. If $x = 6.5$ weeks and $k = 20$ eggs for 2 weeks in succession, then $r = 0.535$ but the stable age population contains only 1.7% adults 1 week old and 1.0% 2 weeks old. It is thus obvious that the r value for a 2-week laying period must be less than the sum of the 2 weeks considered independently. Similarly λ is not directly additive, but actual egg numbers weighted according to Table 1 and the method described earlier are additive.

In some circumstances, even with long oviposition periods, the primitive single period method of calculating λ gives a good estimate. This is so when the shape of the egg pattern is fairly symmetrical with a pronounced hump near the centre where, according to this method, all the eggs are assumed laid.

Table 3 gives the values for λ (antilog_e r) found by the methods mentioned in this paper, for the examples mentioned here, and shows that, by chance, the single oviposition period method sometimes gives a good estimate of λ . With the single period method the maximum estimate, which is usually the best estimate, can be obtained by cutting off and ignoring all eggs laid after the peak of the oviposition

curve. The maximum may be found more accurately by plotting a cumulative oviposition curve on logarithmic graph paper in the style of curve *A* of Fig. 1.

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STUDIES ON SYSTEMIC FUNGICIDES

III. THE ACTIVITY OF CERTAIN CHLORINE-SUBSTITUTED β -NAPHTHOLS AND NAPHTHYLOXY-*n*-ALIPHATIC CARBOXYLIC ACIDS AS SYSTEMIC FUNGICIDES

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(With 2 Text-figures)

A number of mono-, di- and trichloro- β -naphthols and the corresponding naphthyloxy-*n*-carboxylic acids were prepared. They were tested as systemic fungicides against chocolate spot of broad bean (*Vicia faba*) caused by *Botrytis fabae* and as fungicides *in vitro* by their effect on the germination of spores of *Sclerotinia laxa* and the growth of the mycelium of *Botrytis fabae*.

The systemic fungicide tests showed no difference in the activity of the compounds associated with different side chains, though there were slight but significant effects associated with different chlorine substitutions in the naphthalene nucleus. The 3:4-dichloro-2-naphthol derivatives were more effective than the other compounds tested.

Fungicide tests with the 1:3-dichloro-2-naphthol derivatives suggested that there was some increase in toxicity to the spores of *Sclerotinia laxa* with increasing length of side chain. The effectiveness of the various chlorine-substituted compounds in suppressing growth of *Botrytis fabae* was in the following descending order, 1:3:4-trichloro-, 1:3-dichloro-, 1:4-dichloro- and 3:4-dichloro-compounds. The concentrations required in agar culture to induce reductions of *B. fabae* growth comparable to those found in the leaf could reasonably be expected to occur in the bean plants treated with the 2-naphthyloxyacetic acids at 10 p.p.m.

INTRODUCTION

It has been suggested (Crowdy & Wain, 1951) that a logical approach to the study of systemic fungicides would be provided by examining chemicals known to be translocated within the higher plants and testing the more fungicidal of these for their ability to check disease when distributed systemically. The compounds used must not induce abnormal growth in the host. Many of the aryloxyalkylcarboxylic acids are known to be translocated within higher plants, and the fungitoxicity of a number of these acids has been assessed by their ability to reduce the growth of *Nectria galligena* Bres. on agar (Crowdy, 1948). These tests, in which the fungus was grown on malt agar containing 100 p.p.m. of the acid, indicated that the naphthyloxy acids were more toxic than the phenoxy acids, and that introduction of chlorine into the aryl part of the molecule enhanced toxicity.

Crowdy & Wain (1951), using chocolate spot of broad bean (*Vicia faba* L.),

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caused by *Botrytis cinerea* Pers. and *B. fabae* Sardiña, demonstrated a marked reduction in the disease on the leaves following root application, stem injection or leaf spraying with a number of phenoxy acids, particularly 2:4:6-trichlorophenoxy-acetic, pentachlorophenoxyacetic and pentachlorophenoxy-*isobutyric*. The following naphthyloxy acids were also included in these trials:

β -(1-naphthyloxy)propionic acid,
 β -(2-naphthyloxy)propionic acid,
 β -(1-chloro-2-naphthyloxy)propionic acid,
 1:4-dichloro-2-naphthyloxy acetic acid,
 α -(2-naphthyloxy)phenylacetic acid.

Of these, only the last was recorded as producing a reduction in chocolate spot symptoms and even here the performance was erratic. There is evidence from plant hormone studies that the naphthyloxy acids will enter the higher plant (Zimmerman & Hitchcock, 1941), and it seems possible that the failure of these compounds may be due to their physiological effects on the host since all, except possibly the first, have been reported to induce abnormal growth effects (Osborne, Wain & Walker, 1952). The present trials describe an examination of a number of substituted naphthyloxy acids as fungicides *in vitro* and as systemic fungicides against chocolate spot of bean.

MATERIALS

The compounds used in this investigation consisted of several series of substituted 2-naphthyloxy aliphatic carboxylic acids. The substituted acetic acids shown in Table 1 have been previously reported (James & Woodcock, 1951*a, b*) having been originally prepared for examination as parthenocarpic agents.

TABLE 1. *Substituted 2-naphthyloxy acetic acids*

Nuclear substituent	Melting-point (°)	Found† (Cl %)	Formula	Requires (Cl %)
3-Chloro-	182-183	15.4	C ₁₂ H ₉ O ₃ Cl	15.0
4-Chloro-	168-169	14.4	C ₁₂ H ₉ O ₃ Cl	15.0
7-Chloro-	168-169	14.7	C ₁₂ H ₉ O ₃ Cl	15.0
1:3-Dichloro-	179-180	25.8	C ₁₂ H ₈ O ₃ Cl ₂	26.2
1:4-Dichloro-	188-189	25.9	C ₁₂ H ₈ O ₃ Cl ₂	26.2
3:4-Dichloro-	182-184	25.7	C ₁₂ H ₈ O ₃ Cl ₂	26.2
1:3:4-Trichloro-	207-208	34.7	C ₁₂ H ₇ O ₃ Cl ₃	34.8

† Chlorine analyses (Robertson's method) by R. F. Batt, of this Department.

The α -substituted acids were obtained by the following general procedure, analytical details being given in Table 2. An ethyl alcoholic solution of the naphthol was treated with sodium ethoxide (1 mol.) and the solution heated for several hours under reflux with the appropriate α -bromo ester (1 mol.). After the addition of excess 10% aqueous sodium hydroxide solution the mixture was heated for a

further 1 hr., cooled and acidified with concentrated hydrochloric acid. The product was isolated with ether and obtained free from the naphthol, by extraction of the ethereal solution with saturated sodium hydrogen carbonate. The aqueous layer was washed with ether, separated and acidified with concentrated hydrochloric acid, the precipitated acid being isolated by re-extraction with ether. After drying the ethereal extract over anhydrous sodium sulphate, the solvent was distilled off and the residual acid was recrystallized to constant melting-point.

TABLE 2. *Substituted α -(2-naphthyloxy)-n-aliphatic carboxylic acids*

Nuclear substituent	Side chain	Melting-point (° C.)	Found† (Cl %)	Formula	Requires (Cl %)
1-Chloro-†	—O.CH(CH ₃)COOH	171–172	14·1	C ₁₃ H ₁₁ O ₃ Cl	14·2
3-Chloro-	—O.CH(CH ₃)COOH	175–176	14·1	C ₁₃ H ₁₁ O ₃ Cl	14·2
8-Chloro-	—O.CH(CH ₃)COOH	132–133	14·0	C ₁₃ H ₁₁ O ₃ Cl	14·2
1:3-Dichloro-	—O.CH(CH ₃)COOH	131–132	24·7	C ₁₂ H ₁₀ O ₃ Cl ₂	24·9
1:4-Dichloro-	—O.CH(CH ₃)COOH	160–161	24·4	C ₁₃ H ₁₀ O ₃ Cl ₂	24·9
3:4-Dichloro-	—O.CH(CH ₃)COOH	176–177	25·5	C ₁₃ H ₁₀ O ₃ Cl ₂	24·9
1:3:4-Trichloro-	—O.CH(CH ₃)COOH	163–164	33·0	C ₁₃ H ₉ O ₃ Cl ₃	33·3
3-Chloro-	—O.CH(C ₂ H ₅)COOH	149–150	13·1	C ₁₄ H ₁₂ O ₃ Cl	13·4
1:3-Dichloro-	—O.CH(C ₂ H ₅)COOH	81–82	23·7	C ₁₄ H ₁₂ O ₃ Cl ₂	23·7
1:4-Dichloro-	—O.CH(C ₂ H ₅)COOH	113–114	23·1	C ₁₄ H ₁₂ O ₃ Cl ₂	23·7
3:4-Dichloro-	—O.CH(C ₂ H ₅)COOH	153–154	23·6	C ₁₄ H ₁₂ O ₃ Cl ₂	23·7
1:3:4-Trichloro-	—O.CH(C ₂ H ₅)COOH	120–121	31·5	C ₁₄ H ₁₁ O ₃ Cl ₃	31·9
3-Chloro-	—O.CH(C ₃ H ₇)COOH	159–160	13·0	C ₁₅ H ₁₅ O ₃ Cl	12·8
1:3-Dichloro-	—O.CH(C ₃ H ₇)COOH	97–98	22·1	C ₁₅ H ₁₄ O ₃ Cl ₂	22·7
3-Chloro-	—O.CH(C ₄ H ₉)COOH	127–128	12·4	C ₁₆ H ₁₇ O ₃ Cl	12·1
1:3-Dichloro-	—O.CH(C ₄ H ₉)COOH	101–102	21·2	C ₁₆ H ₁₆ O ₃ Cl ₂	21·7

† Chlorine analyses (Robertson's method) by R. F. Batt, of this Department.

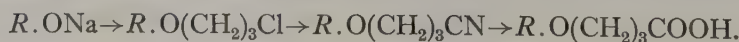
‡ Previous reference for this substituent is found in Osborne *et al.* (1952).

TABLE 3. *Substituted ω -(2-naphthyloxy)aliphatic carboxylic acids*

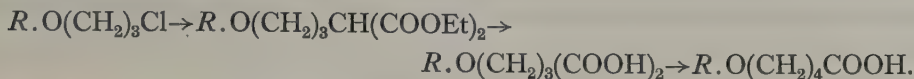
Nuclear substituent	Side chain	Melting-point (° C.)	Found† (Cl %)	Formula	Requires (Cl %)
1:3-Dichloro-	—O.(CH ₂) ₃ COOH	107–108	24·9	C ₁₃ H ₁₀ O ₃ Cl ₂	24·9
1:3-Dichloro-	—O.(CH ₂) ₃ COOH	85–86	23·0	C ₁₄ H ₁₂ O ₃ Cl ₂	23·7
1:3-Dichloro-	—O.(CH ₂) ₄ COOH	62–63	21·6	C ₁₆ H ₁₄ O ₃ Cl ₂	22·7
1:3-Dichloro-	—O.(CH ₂) ₅ COOH	81–83	20·9	C ₁₆ H ₁₆ O ₃ Cl ₂	21·7

† Chlorine analyses (Robertson's method) by R. F. Batt, of this Department.

The ω -substituted acids are listed in Table 3. Condensation of β -propiolactone with the sodium salt of the appropriate naphthol gave β -substituted propionic acids in a manner similar to that described by Gresham, Jansen, Shaver, Bankert, Beears & Prendergast (1949). γ -Substituted butyric acids were synthesized by the following route, trimethyl chlorobromide being employed for the initial condensation:



Reaction of the chloro-propanes obtained in this way with diethyl sodiomalonate gave a route to δ -substituted valeric acids according to the scheme:



ϵ -Substituted caproic acids were obtained, using 1:5-dibromopropane, by the same route as used for the γ -butyric acids. The formation of the doubly condensed products $R.O(CH_2)_5O.R$ was kept to a minimum by the dropwise addition of an ethyl alcoholic solution of the sodio-naphthol, this being added to an excess of the dibromide in boiling ethyl alcoholic solution.

The biological activity of this series of substituted 2-naphthyloxy aliphatic carboxylic acids and of the corresponding naphthols has been investigated both as systemic fungicides and as fungicides *in vitro*.

EXPERIMENTAL METHODS

The effect of these compounds as systemic fungicides has been assessed on their ability to reduce chocolate spot of the broad bean caused by *B. fabae*. A detailed description of this test has already been published (Crowdy & Wain, 1951). Broad beans which had been sown in potting soil were lifted when the plants had reached a suitable size and after their roots had been washed, they were transferred to water or nutrient solution containing the compound under test. After varying periods of treatment the plants were sprayed with a spore suspension of *B. fabae* in 1% sucrose and incubated in a saturated atmosphere at approximately 70° F. for 2 or 3 days. The full period of the test varied from 10 to 21 days; nutrient solutions being used only in the long-period trials. The disease was assessed by measuring the diameters of ten lesions on each of the three or four topmost leaves with a micrometer eyepiece in a low-power microscope. No attempt was made to standardize the spore suspension accurately from one trial to the next, but a uniform rate of inoculation was provided within the trials by spraying the plants to run-off with the same spore suspension. The square of the mean lesion diameter was taken as a measure of the percentage area of the leaf which was diseased. Some estimate of the damage to the bean plants was obtained by measuring the heights of the plants at the end of the trials.

There was considerable variation in the rate of disease development within the incubation chambers, and different environmental conditions during the period of treatment probably affected the uptake of the compounds under trial. Allowance was made for these effects by replicating the treatments and repeating the trials at different times.

In most experiments the concentrations of the acids used were calculated on a weight:volume basis, but in some a convenient concentration of the dichloro-naphthyloxybutyric acid was chosen and the other acids were tested at equivalent

molecular concentrations. In practice it was found that 1, 10, and 25 p.p.m. all produced approximately the same reduction in disease, and that the relatively minor adjustments in weight required to give equimolecular concentrations did not have enough effect to justify the added complications in testing procedure. Most of the tests were carried out at 10 p.p.m.

In addition to the water culture tests, beans were raised in soil containing the selected compounds, at a concentration of approximately 50 p.p.m. These plants were inoculated and the effect of treatment on disease was assessed by the method described above.

The fungitoxicity of the compounds *in vitro* was assessed by their ability to inhibit spore germination and their effect on the growth rate of the fungus on agar plates. In the spore germination tests the test-tube dilution technique recommended by the American Phytopathological Society's Committee on standardization of fungicidal tests (1947) was employed. Spores of *Sclerotinia laxa* Aderh. & Ruhl. were obtained from 7-day cultures grown on autoclaved potato plugs previously soaked in 7.5 % malic acid for 3 days. A sucrose-sodium citrate mixture was used as spore stimulant at concentrations of 0.1 and 0.001 % respectively. The final spore concentration was approximately 50,000 per millilitre. The germination of 100 spores was recorded after incubation for 20 hr. at 25° C. The germination data were transformed to probits and plotted against the logarithm of the dosage in the conventional manner. The dosage ratio used was $\sqrt{2}$ throughout the tests.

The effect of the compounds on the growth of the fungus on agar was assessed by a variation of the method described by Crowdy (1948). The fungus used throughout was *B. fabae* and the same strain was used in both the systemic and *in vitro* fungicide tests. The *B. fabae* was grown on a phosphate-dextrose-peptone medium, the pH of which was adjusted to 4.4 or 4.5 by the addition of N/10-sulphuric acid. The compounds under test were added to the medium at 45–50° C. from an ethyl alcoholic solution, the concentration of which was adjusted to give a final ethyl alcohol concentration in the medium of 1 %. The growth of each colony was estimated as the mean of two diameters at right angles to each other measured at varying periods. It was demonstrated that, after a short initial lag period, the daily increase in diameter was constant until the drying out of the agar concentrated the compound in the medium and induced a reduction in growth rate. In practice the time taken for the test was reduced to a minimum to avoid this concentration effect, and the average growth rate was estimated between the third and fifth days after inoculation.

ACTIVITY AS SYSTEMIC FUNGICIDES

The investigations were confined to a number of substituted β -naphthols and the derived naphthylxyaliphatic acids in which the side chain had been varied and chlorine atoms introduced into the nucleus.

Monochloro acids

A number of monochloronaphthyloxy acids were included in preliminary trials namely:

- 3-, 4- and 7-chloro-2-naphthyloxyacetic acids,
- 1-, 3- and 8-chloro-2-naphthyloxy- α -propionic acids,
- α -(3-chloro-2-naphthyloxy)-*n*-butyric acid,
- α -(3-chloro-2-naphthyloxy)-*n*-valeric acid,
- α -(3-chloro-2-naphthyloxy)-*n*-caproic acid.

All showed some significant reduction in disease in the treated plants, but usually the reduction was significantly less than that recorded with 2:4:6-trichlorophenoxyacetic acid treatment. The reduction in disease induced by the 3-chloro-acids was comparable with that induced by 2:4:6-trichlorophenoxyacetic acid and these compounds were tested as soil treatments. The prolonged test in soil revealed that the acids in this series all produced marked growth effects, shown as a mosaic and frenching of the leaves and a stunting of growth which was marked in the case of the acetic and the α -propionic acids. The obvious morphogenic effects rendered these acids unsuitable for further trials.

Di- and trichloro-2-naphthols and 2-naphthyloxy acids

The dichloro- and trichloro-2-naphthyloxy acids and the corresponding naphthols which were tested in water culture are listed in Table 4. None of these acids showed formative effects but they were all liable to cause phytotoxic damage. At 50 p.p.m. 1:3-dichloro-2-naphthyloxyacetic acid, the behaviour of which was typical of this group excluding γ -(1:3-dichloro-2-naphthyloxy)-*n*-butyric acid, induced a severe wilting of the leaves and a blackening of the vascular tissue which was visible on the surface of the stems of the treated plants. At 25 p.p.m. the

TABLE 4. *Di- and trichloro-2-naphthols and 2-naphthyloxy-n-aliphatic carboxylic acids tested as systemic fungicides in water culture*

Side chain	Nuclear chlorine substitution			
	1:3	1:4	3:4	1:3:4
Hydroxyl	t	t	t	t
Acetic acid	t	t	t	t
α -Propionic acid	t	t	t	t
β -Propionic acid	t	—	—	—
α -Butyric acid	t	t	t	t
γ -Butyric acid	t	—	—	—
α -Valeric acid	t	—	—	—
δ -Valeric acid	t	—	—	—
α -Caproic acid	t	—	—	—
ϵ -Caproic acid	t	—	—	—

t = compounds tested at 1 and 10 p.p.m.

wilting was slight and the plants recovered in the saturated atmosphere of the inoculating chamber. At these concentrations root damage was severe. At 10 p.p.m. there were no obvious damage effects in the tops of the plants but the roots were poorly developed. Treatment with all these compounds at 10 p.p.m. caused a reduction in height (approximately 25%) and this was severe in plants treated with γ -(1:3-dichloro-2-naphthyl)-*n*-butyric acid; these were only about half the size of the controls. At 1 p.p.m. obvious signs of damage were absent and the average height was 80–85% of the controls: at this concentration the reduction in height following treatment with γ -(1:3-dichloro-2-naphthyl)-*n*-butyric acid was not significantly greater than that recorded with the other acids.

The systemic fungicidal activity of the β -naphthols with the four different chlorine substitutions, and their derived naphthylacetic, α -propionic and α -*n*-butyric acids (Table 4) was compared in a trial, the results of which are presented in Tables 5 and 6. In this trial the compounds were used at equimolecular concentrations of approximately 10 p.p.m.; the full trial was repeated four times and the compounds were replicated three times in each repetition. The treatment time in each experiment was about 2 weeks.

Table 6 shows that there were significant differences in both lesion diameter and plant height due to treatment. The effect of the side chain on the diameter of the

TABLE 5. *Effect on systemic fungicidal activity and height of bean plants of aliphatic side chain and chlorine substitution of 2-naphthols and derived 2-naphthyl- α -aliphatic acids*

(All figures in mm.)										
Individual means										
Side chains	Hydroxyl		Acetic acid		α -Propionic acid		α -Butyric acid		General means	
	Lesion diam.	Plant height	Lesion diam.	Plant height	Lesion diam.	Plant height	Lesion diam.	Plant height	Lesion diam.	Plant height
Chlorine substituents:										
1:3-Dichloro-	0.917	253	0.841	245	0.877	236	0.953	257	0.897	248
1:4-Dichloro-	0.885	258	0.909	230	0.858	226	0.929	207	0.894	230
1:3:4-Trichloro-	0.905	254	0.929	249	0.858	214	0.850	233	0.884	238
3:4-Dichloro-	0.822	270	0.846	269	0.858	242	0.850	252	0.843	258
General means:										
Lesion diam.	0.881	—	0.881	—	0.861	—	0.894	—	—	—
Plant height	—	259	—	248	—	230	—	237	—	—
Least significant differences										
Probability levels	Diameter				Height					
	Individual means		General means		Individual means		General means			
0.05	0.048		0.024		35		17			
0.01	0.062		0.031		45		22			

lesions just failed to reach the conventional 5 % level of significance, but the effect of chlorine substitution was highly significant: the lesions formed on the plants treated with the 3:4-dichloro-compounds were smaller than those on the plants treated with the other three series of compounds which did not differ from each other in diameter. The plants treated with the naphthols were significantly taller than those treated with either the α -propionic or the α -*n*-butyric acids, while those

TABLE 6. *Analysis of variance applied to the effects shown in Table 5*

Effect	Lesion diameter		Plant height	
	Degrees of freedom	Variance	Degrees of freedom	Variance
Side chain	3	0.252	3	7900**
Chlorine substitution	3	0.884***	3	7067**
Interaction:				
Side chain \times chlorine substitution	9	0.510**	9	1522
Error	5709	0.104	144	1806

** Significant difference, $P=0.01$.

*** Significant difference, $P=0.001$.

treated with the acetic acids were intermediate in size. The plants treated with 3:4-dichloro-compounds were significantly taller than those treated with either the 1:4-dichloro- or 1:3:4-trichloro-compounds and those treated with the 1:3-dichloro-compounds were intermediate in size. The fact that the 3:4-dichloro-substituted compounds are the least damaging to the host and also the most effective in reducing the disease suggests the possibility that damage to the host and disease reduction may be related. The data did not allow a precise examination of this effect, but it is interesting to note that the 3:4-dichloro-2-naphthol, the compound which one would expect to be least phytotoxic actually gave the tallest plants and the smallest lesions. This trial also illustrates that the naphthols were as effective as the naphthyloxy acids as systemic fungicides.

The tests included a greater range of 1:3-dichloro-2-naphthyloxy-*n*-aliphatic acids than the other chlorinated acids (see Table 4), but usually the response of the disease was unaffected by the aliphatic part of the molecule. There was little difference in response between plants treated at 10 and at 1 p.p.m. An exception to this generalization was the γ -(1:3-dichloro-2-naphthyloxy)-*n*-butyric acid which was markedly more effective at 10 p.p.m., and at 1 p.p.m. produced the same order of disease reduction as the others: the effect of this compound on the host, which has been mentioned above, was also exceptional.

It has been suggested (Keyworth & Dimond, 1952) that damage to the roots of treated plants had a significant effect in reducing *Fusarium* wilt of tomato. It was felt that if a phytotoxic effect of this type was operating in these trials, it might show as a positive correlation between the height of the plants and the mean diameter of the lesions formed on them. This correlation was calculated for the

series of plants treated with 1 p.p.m. of the acids and gave a coefficient of only 0.042 calculated from seventy-seven pairs of observations showing that no effect of this type could be demonstrated with this series.

The effect of soil treatment with the following compounds was also investigated: 1:3-dichloro-2-naphthol, 1:3-dichloro-2-naphthyloxy acetic, γ -butyric, δ -valeric and ϵ -caproic acids, and 2:4:6-trichlorophenoxyacetic acid. The soil in the pots was treated to give a concentration of approximately 50 p.p.m. of the acids, and the beans were sown in the treated soil. In due course the plants were inoculated and the disease assessed in the usual way. After assessment the plants were removed and the same soil was re-sown with a fresh series of beans without further treatment. This process was repeated until the plants grown in the treated soil showed no marked reduction in disease. In the first assessment the plants treated with γ -(1:3-dichloro-2-naphthyloxy)-*n*-butyric acid showed less disease than those treated with the other naphthol derivatives, but this effect was not apparent in the later trials: this result would be expected from the dilution effect shown in the water-culture tests. Apart from the effect of this acid in the first trial, there were no consistent differences in the naphthyloxy series. The results of this trial are summarized in Table 7, in which the effects of these treatments have been grouped.

TABLE 7. *Soil treatment with 1:3-dichloro-2-naphthol, a series of 1:3-dichloro-2-naphthyloxy-n-aliphatic acids and 2:4:6-trichlorophenoxyacetic acid*

Date of assessment	Percentage reduction in disease					
	... 12. vii. 51	14. viii. 51	14. ix. 51	29. x. 51	7. xii. 51	1. ii. 52
Naphthol derivatives	26	28	14	31	24	2
2:4:6-Trichlorophenoxy acetic acid	33	23	29	26	29	19

A single initial treatment given on 18 June 1951.

It can be seen from this table that the effect of treatment in these conditions will persist for about 6 months and during this period the level of control is approximately constant. In this trial the phenoxy acid appeared to be rather more effective than the naphthyloxy acids in reducing the disease. It is only reasonable to assume that the acid content of the soil was reduced with each successive planting and the fact that this reduction was not reflected in the disease reduction figures confirms the observations on the dilution of the active principle in the water-culture trials. A similar failure to respond to changes in concentration has been recorded with a number of phenoxy acids (Crowdy & Wain, 1951).

IN VITRO ACTIVITY AS FUNGICIDES

The results of the spore germination tests with the chlorinated naphthols and the chlorinated 2-naphthyloxy aliphatic acids listed in Table 4 showed that the slopes of the dosage-response lines varied with different substitutions in the naphthalene

nucleus. In these circumstances it was only possible to compare the relative potencies of the acids in one particular series. There was little to choose between the series from a fungicidal point of view, and the detailed observations were confined to 1:3-dichloro-2-naphthol and the 1:3-dichloro-2-naphthyloxyaliphatic acids, for which parallel dosage-response lines were obtained. The results of these tests are listed in Table 8: in this table the relative potencies have been expressed both on a weight/volume and on a molar basis. ED-50 values were of the order of 20 p.p.m. The variations in toxicity were not wide, but there appeared to be some increase of toxicity with increasing length of side-chain.

TABLE 8. *The relative potencies of 1:3-dichloro-2-naphthol and a number of 1:3-dichloro-2-naphthyloxy-n-aliphatic acids*

Compound	Relative potencies	
	Weight/volume	Molar
Naphthol	1.38	1.03
Acetic acid	0.80	0.76
α -Propionic acid	1.00	1.00
α -Butyric acid	0.93	0.98
α -Valeric acid	1.26	1.38
α -Caproic acid	1.26	1.45
β -Propionic acid	1.44	1.44

In studying the growth of *B. fabae* on agar, an attempt was made to determine the effect of varying the concentration of the compounds under test. The response of the fungus to graded doses of the toxicant could be represented by a sigmoid curve, and this curve, like many others of the same type, was reduced to a straight line by transformation to the probit/log. dose form. Two representative lines which have been treated in this way are illustrated in Fig. 1. Up to the present the accuracy of this method of testing, due in part to the low fungitoxicity and the relative insolubility of many of the compounds, does not allow detailed comparisons of relative toxicities of the compounds under investigation. However, a number of points of general interest have emerged. Within the limits of accuracy of the trials neither the chlorine substitution in the naphthalene nucleus nor the constitution of the side chain affects the slope of the probit/log. dose line. There is some indication that the position of the line is affected by the substitution in the nucleus and not consistently affected by the side chain. The order of toxicity for different substitutions in the nucleus is as follows: most toxic, 1:3:4-trichloro-, intermediate and of approximately the same toxicity, 1:3- and 1:4-dichloro-, and least toxic 3:4-dichloro-2-naphthol derivatives.

While it is clearly not possible to make detailed comparisons between the behaviour of the pathogen growing in the living bean tissue and its growth on agar medium, some general observations are of interest. Fig. 2 illustrates theoretical dose/growth inhibition curves for 1:3:4-trichloro-, 1:3- and 1:4-dichloro- (which are

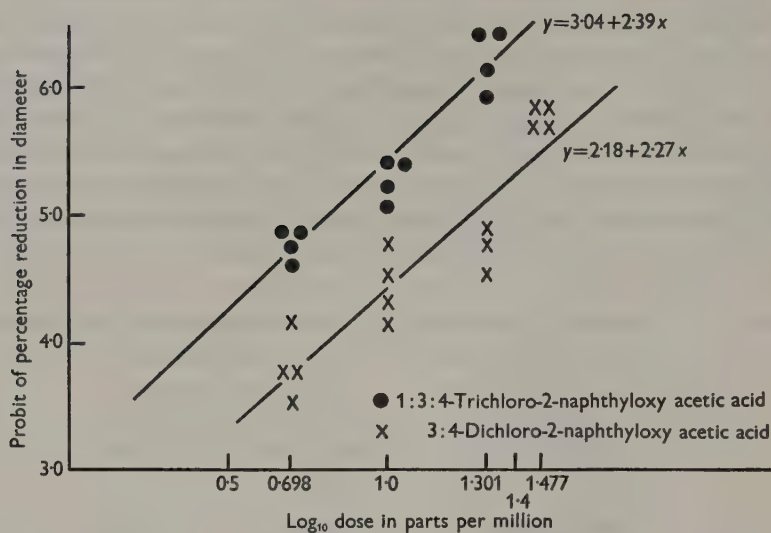


Fig. 1. Probit of percentage reduction in colony diameter/log. dose curves for *B. fabae* on agar medium.

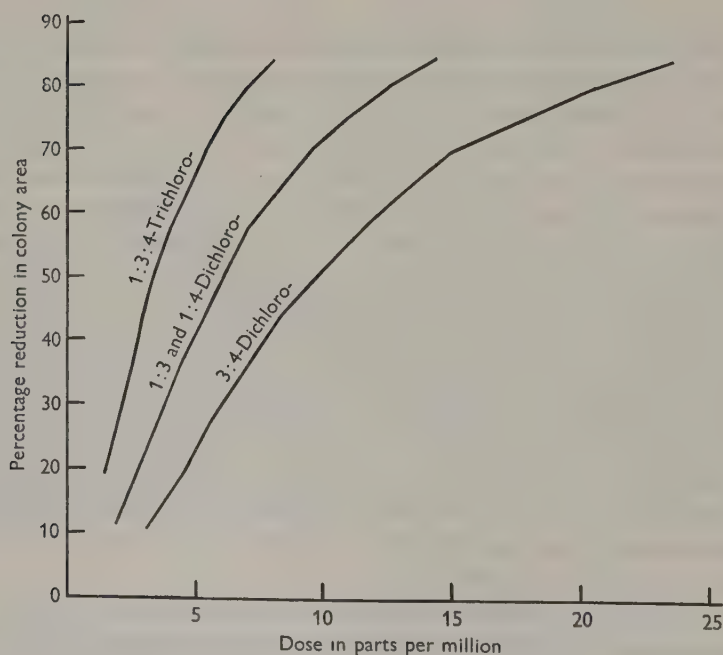


Fig. 2. Percentage reduction in colony area/dose curves for *B. fabae* on agar medium containing chlorine substituted 2-naphthyloxyacetic acids.

represented by the same line), and 3:4-dichloro-2-naphthyloxyacetic acids calculated from the probit/log. dose lines obtained for these four compounds. The growth-inhibition data have been presented as a percentage reduction in colony area calculated from the square of the diameter on the assumption that the colonies are circular. The percentage inhibition calculated in this way is to some extent comparable to the figures for the percentage area diseased used in assessing the effect of the compounds as systemic fungicides. From this figure it is possible to make estimates of the concentrations of acids required to effect specific reductions in colony area. Table 9 lists the concentrations required to effect the reductions in area which were recorded in the systemic fungicide trial described in Table 5.

TABLE 9. *Concentrations of 2-naphthyloxyacetic acids required to effect specific reductions in the growth of Botrytis fabae in culture*

Naphthyloxyacetic acid	Percentage reduction in area	Concentration (p.p.m.)
1:3-Dichloro-	20	2.75
1:4-Dichloro-	20	2.75
1:3:4-Trichloro-	22	1.50
3:4-Dichloro-	29	5.75

It can be seen from Table 9 that the concentrations of the acids required to induce comparable reductions in growth on agar and in the plant are of approximately the same order. The concentrations recorded could reasonably be expected to occur in plants treated with solutions of 10 p.p.m.

DISCUSSION

The tests described above for systemic fungicidal activity consist essentially in treating a plant and measuring the effect of treatment on the development of a particular disease. The actual mechanism of disease reduction is a matter for speculation in the light of collateral evidence. An attempt has been made in the present trials to demonstrate that the existing evidence is not incompatible with the view that the substituted 2-naphthols and 2-naphthyloxy-*n*-aliphatic carboxylic acids reduce disease primarily by affecting the growth of the pathogen.

The testing procedure has a number of defects, many of which might be avoided by a rigid standardization of experimental conditions. It is clear that the environment during growth will affect the metabolism of the host and thus influence the uptake and accumulation of the systemic fungicide under test, and that the extent of disease development will be greatly influenced by conditions at the time of inoculation and incubation. Errors from these external sources will be further aggravated by the inherent variability of the host plant. Some measure of the effect of environment during the treatment period is given by the behaviour of the

same compounds in parallel trials conducted at different times. The effect of environment during disease development can be demonstrated with uniformity trials within the inoculating chambers. Variations between individual plants in these trials may be of the same order as the systemic fungicidal effects measured: variation from this cause may be serious when the data are standardized against the performance of a single treatment, since an abnormally high or low value in the standard will weight the entire series of readings. The widest range of variation was recorded in a repeated series of trials with 1:3-dichloro-2-naphthol derivatives at 10 p.p.m. in which the maximum control was 49% and the minimum 17%. In designing the trials the treatments were replicated to give a measure of the variation in disease development, and the trials were repeated at different times to allow for variations in the external conditions during the treatment period and also for any weighting due to abnormal variations in the standard treatment.

The systemic and *in vitro* fungicide tests revealed little difference between the behaviour of the dichloro- and trichloro-2-naphthol derivatives examined, though the 3:4-dichloro-compounds were slightly better as systemic fungicides and the trichloro-compounds were rather more effective *in vitro* as fungicides. This confirms earlier observations on compounds of this type (Crowdy, 1948). Certain points of interest arise when the data from the systemic and *in vitro* trials are compared with each other and with the data on phytotoxicity. In general, Table 9 shows that the concentrations used in the systemic tests are of the order required to produce the reductions in the growth of the pathogen which have been recorded. Even the highest control value of 49% would require only 6 p.p.m. of the 1:3-dichloro-2-naphthyloxyacetic acid, and these plants were growing in solutions containing 10 p.p.m.

The 3:4-dichloro-2-naphthyloxy derivatives were the least toxic to the pathogen *in vitro*, but in spite of this were the most effective systemic fungicides. This anomalous behaviour requires further investigation. If the results of the observations on phytotoxicity can be taken as a fair measure of the tolerance of the host for the different compounds, an explanation is provided for the failure of dilution of the solutions in the systemic tests to produce a corresponding reduction in the degree of control. Thus if a treatment of 10 p.p.m. can produce an effective concentration in the host of only about 2 p.p.m., it is possible that a concentration in the host of much the same order might result from a treatment of only 1 p.p.m. This type of argument can only be applied to the low concentration ranges; at higher concentrations phytotoxicity becomes serious and the condition of the host becomes a controlling factor.

Comparison of the fungitoxicity and phytotoxicity data in this series of compounds suggests that these two effects are linked, and increases in fungitoxicity are unlikely to result in more effective systemic fungicidal action unless modifications can be made in the molecule which confer greater specificity of action.

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WHITE ROT (*SCLEROTIUM CEPIVORUM*) OF ONIONS IN WORCESTERSHIRE WITH SPECIAL REFERENCE TO CONTROL BY SEED TREATMENT WITH CALOMEL

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Observations in Worcestershire have shown that heavy losses of salad onions due to white rot may occur on sites which have not been known to carry a previous susceptible crop. Reasons for this are discussed. During five seasons a good control of the disease has been obtained by treating the seed with pure calomel applied with a resin-alcohol sticker. The treatment also gave some control of damping off of seedlings due to *Corticium solani*. Broadcasting 4% calomel dust at 2½–3 cwt. per acre was less satisfactory in controlling white rot. In one season promising results were obtained with seed treatment of bulb onions.

OCCURRENCE AND DISTRIBUTION OF THE DISEASE

Onion white rot has long been an important disease of onions in Worcestershire, especially in the intensive market-gardening area of the Vale of Evesham. Except during the war years when policy necessitated growing bulb onions, the crop is principally green or salad onions. The variety grown is almost exclusively White Lisbon which is very susceptible to white rot. The main crop is sown in July or August for harvesting the following spring, though a smaller acreage is sown in spring for harvesting the same summer. The seeding rate is high, usually about 30 lb. per acre, sometimes up to 40 lb. There is thus a dense stand of plants, and once the disease is established it spreads rapidly under favourable conditions. In the July-sown crop the disease may occur in October to November causing a serious thinning out of the plants in large patches. A second attack may develop in the spring, often in dry weather just prior to harvesting, causing in some cases almost total destruction of the crop. Losses may occur at any time in spring-sown crops but are most frequent in June and July. The disease occurs on all soil types on which the crop is grown from the light sandy, glacial drifts of the Pershore and Crophorne Heath Series to the lias clays of the Evesham and Haselor Series. It is found equally on acid and alkaline soils, severe attacks having been noted on fields of pH 6·5 and 7·8. In some areas of the county market gardeners have given

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up growing the salad onion on some of their ground owing to frequent losses while others, especially on the lighter soils, have attempted, not always successfully, to reduce their losses by early sowing and marketing.

The disease is not confined to areas where onions have been grown frequently. Some of the worst outbreaks causing losses of over 90% of the crop have occurred on land carrying the first known onion or leek crop. This occurred on a number of occasions during the war following the ploughing up of grassland or the grubbing of derelict orchards. These outbreaks still present an unsolved problem. The possibility of seed-borne infection, as suggested by Moore (1944), has been considered, but the occurrence of the disease in some fields in rectangular patches at right angles to the line of drilling does not support this view. The fact that on several occasions seed from the same sample was sown on adjacent land where no white rot appeared also opposes this theory. A careful examination was made of nearly 100 samples of seed without finding any infection present, and it seems unlikely that enough infected seed or sclerotia could be present in a sample to cause an almost complete loss of crop. As reported by Moore (1944) the disease may infect the wild onion *Allium vineale*. This might account for some of these outbreaks, but in others this weed was not found on the site nor seen on that soil type. There was no evidence of the disease being brought on to the land by tools or on the soil clinging to seedling roots of a previous crop transplanted on to the ground. In one case a grower bought a paddock which had been down to grass for over twenty years, and incorporated it into his adjacent arable holding. After two brassica crops the newly broken area and part of the adjoining land were sown with salad onions. On the area which was previously grass the crop was destroyed by white rot while the adjacent area which had carried onions biennially for many years was entirely free from the disease. Such cases suggest that either this fungus has a wider host range than the onion family or that it exists as a saprophyte in the soil. Although careful search has been made on weeds in infected crops no sign of the disease has been seen. Even leeks are comparatively rarely attacked by the fungus, though grown on the same sites as onion and at a similar time of the year. Until more is known of the persistence of the organism in the soil it is evident that one cannot recommend with confidence a longer interval between onion crops as a control measure.

EXPERIMENTAL

Method of treating seed

The experiments of Booer (1945, 1946) encouraged the writers to investigate the use of calomel for controlling white rot in Worcestershire. The method successfully used by him of applying 1 lb. of 4% calomel dust to 50 yd. of seed row before drilling was not applicable on a field scale as no machine existed that would carry out the operation. Broadcasting the dust before sowing had not proved as effective nor is this an easy process to carry out uniformly on the field scale. We therefore

returned mainly to the method of using the seed as vehicle for putting the fungicide in the soil, although Boorer (1945) found this less effective than soil treatment. A number of stickers were tested in order to obtain good adherence of pure calomel to the seed. These included water paste (Wright, 1939), methyl cellulose (Newhall, 1945), 24% resin dissolved in hot potash; and resin-alcohol solution containing 26.6% by weight of resin in industrial methylated spirit, sp.gr. 0.825. Both resin materials were effective, but the resin alcohol material was chosen for most of the experiments as the material was cheap, easily prepared, and dried quickly. Different amounts of sticker were also tested.

The resultant method of treating the seed was as follows: for each pound of seed one fluid ounce of sticker was added and shaken for half a minute to wet all the seed. The necessary amount of pure calomel was then added and shaken until it all adhered to the seed. The seed was then immediately spread out in a thin layer to dry. Occasionally all the calomel did not adhere and then the dried seed was retreated with a fresh dose of sticker and the remaining calomel added and re-shaken. In this way loads of calomel equal to the weight of the seed were obtained and were drilled successfully through the brush drills popular in the area with little loss of fungicide. By re-treating seed several times even greater loads of dust were stuck to the seed. Germination tests showed that there was no reduction of viability following treatment unless the seed was not dried before replacing in a bag. Treated dry seed has been stored for a year without loss of viability compared with untreated seed. Unless otherwise stated, the resin-alcohol sticker was used throughout the following experiments.

Field trial 1

The first trial was laid down in May 1945 at Harvington on ground where an onion crop had been completely destroyed by white rot in the spring. The treatments used were: (A) 1 lb. 4% calomel to 50 yd. of drill; (B) 2 lb. 4% calomel to 50 yd. of drill; (C) pure calomel at same weight as seed applied with resin-potash sticker; and (D) a dust containing tetrachlor-nitro-benzene at the same rate and applied with the same sticker as the calomel. The last-named material was used as a private communication from a Dutch grower suggested that this material had been successfully used in Holland. The seed treated with this compound failed almost entirely to emerge. The calomel treatments gave as good an emergence as the untreated control, though 2-3 days later. No white rot developed in any plot.

Field trial 2

A second trial was sown in July 1946 at Church Lench on a lias clay soil on which the occupier had given up onion growing because of this disease. The only treatment was seed treatment with the same weight of calomel as seed applied with resin potash sticker. The variety was White Lisbon, and there were nine pairs

of treated and untreated rows sown with a brush drill between the rows of a young asparagus bed. The seeding rate was 35 lb. per acre. Germination was uniformly good and there was a good stand during August and September. White rot was noted early in October and continued to develop during that month. After the first week in November until harvesting the following March no further spread occurred. Counts were made on 31 October of numbers of infected and healthy seedlings in five 1 yd. lengths taken at random in each row. The results are given in Table 1. The differences are highly significant, and as no other disease was seen it seems likely that they are due to control of white rot.

TABLE 1. *Effect of seed treatment on development of white rot*

(Mean numbers of plants per 1 yd. of drill.)

	Treated	Untreated	S.E.
Healthy	52.0	29.3	3.21
Infected with white rot	0.2	11.0	0.87

Field trial 3

This trial was laid down in July 1947 in a market garden at Birlingham on a light sandy soil which again had a long history of this disease. The treatments were:

- (1) Control—no treatment.
- (2) Soil treated with $2\frac{1}{2}$ cwt. per acre of 4% calomel broadcast before sowing.
- (3) Seed treated with equal weight of calomel using resin alcohol sticker. ✓
- (4) Seed treated as in (3) sown on soil treated as in (2).

There were three replicates of each treatment. There were no significant differences in population determined by counting 1 yd. samples in each treatment, but the seedlings from treated seed were somewhat retarded compared with the remainder. No white rot appeared in the autumn but counts in December gave consistently about five onions per yard more in the drills sown with treated seed than in those with the other treatments. Lifting began in mid-March and the seed coats of the treated seed were still partly covered with calomel. Only occasional infected plants were then seen, but a rapid spread of the disease occurred at the end of the month. By then only one replicate remained to be harvested. On 1 April ten counts were made of healthy and diseased plants in 1 yd. lengths of drill for each treatment. The results are shown in Table 2. As only one replicate of each treatment remained no statistical analysis is possible, but the result indicates that some control had been obtained particularly by the seed treatment.

Another trial was laid down on adjoining ground the following year but no disease appeared.

Field trial 4

In July 1948 a large-scale field plot was sown on lias clay soil at Haselor. This was on land known to be heavily contaminated with the fungus where a previous

onion crop, the first grown on the site, had failed through white rot. A strip of approximately 2 acres was sown with seed treated with two-thirds its weight of calomel. On each side was drilled approximately 1 acre of untreated seed. The seeding rate of the untreated seed was about 32 lb. per acre and of the treated seed slightly less as no adjustment was made to the drill to allow for the increased size of the treated seed. White rot appeared in October and completely destroyed one

TABLE 2. *Effect of seed and soil treatments on incidence of white rot*

Treatment	Average number of plants per yard of drill	
	Healthy	Infected with white rot
Control	43.5	3.0
Soil treated with 4 % calomel	48.3	1.7
Seed treated with pure calomel	60.0	0.3
Treated seed and soil treated with 4 % calomel	61.2	0.2

area sown with untreated seed. In the remaining areas sown with untreated seed approximately 50 % of the plants were attacked. No diseased plants could be found in the area sown with treated seed. Further development of the disease took place in the following April. Excluding the area destroyed in the autumn, counts were made of numbers of plants in twenty 1 yd. lengths of drill on 3 May. For the treated seed there was an average of 48 healthy and 0.2 diseased plants per yard, and for the untreated 9 healthy and 12 infected plants per yard. Although it was impossible to replicate the plots the results again point to the success of the treatment.

Field trial 5

In 1948 a small plot of old market-garden land adjoining the Evesham laboratory was found to be heavily contaminated with white rot and was therefore used for small-scale field trials to investigate amount of calomel needed as a seed treatment to obtain a control. The following treatments were used:

- (1) Untreated control.
- (2) Calomel at same weight as seed.
- (3) Calomel at three-quarter weight of seed.
- (4) Calomel at one-half weight of seed.

The seed was sown in rows 2 ft. long and 1 ft. apart at a rate equivalent to 28 lb. per acre. There were five replicates of each treatment randomized in blocks.

The seedlings in the treated rows emerged 1-2 days later than those in the control rows and early growth also appeared slower. The difference in height, however, disappeared about 3 weeks after emergence. In early September seedlings were seen to be dying in the untreated rows. Examination of the plants showed that they

were attacked not by white rot but by *Corticium solani*. The result of counts made on 4 October of numbers of seedlings surviving this attack are given in Table 3. Although all treated rows contained more seedlings than control rows the difference is not statistically significant.

No white rot appeared until early April. In order to test the treatments as fully as possible the onions were not harvested until 31 May to 1 June, by which time they had begun to bolt. At this time seed-coats with calomel adhering were still present in the treated rows. All plants were examined and healthy and diseased ones counted. The results are summarized in Table 3.

TABLE 3. *Effect of seed treatments at different rates on control of white rot*

(Mean numbers of healthy and white rot infected plants per yard of drill.)

	Control	Seed treated			S.E.
		Equal wt. calomel	$\frac{3}{4}$ wt. of calomel	$\frac{1}{2}$ wt. of calomel	
No. of healthy seedlings, 4 Oct.	86	113	104	112	16.9
No. of healthy plants, 31 May-1 June	9.3	68.9**	52.4**	46.1**	6.50
No. of white rot infected plants, 31 May-1 June	18.9	4.3**	4.2**	10.3**	1.03

** Significant at 1 % point.

The numbers of healthy plants in every treatment is significantly greater at the 1 % point than the number in the control. The difference between the equal weight and half-weight treatments is significant at the 5 % point. The converse is true of the numbers of diseased plants, indicating that the treatments were controlling white rot disease. In the treated rows infection of the tops by *Botrytis squamosa* and *B. cinerea* caused some loss of plants, the crowded plants in these rows providing more favourable conditions for infection than the sparser population in the control rows.

Field trial 6

A similar trial was carried out on the same site in 1950-51 except that another seed treatment, thiram (tetramethylthiuramdisulphide), was included. The treatments were:

Control, no treatment.

Calomel at same weight as seed (100 % of seed weight adhered).

Calomel at three-quarters weight of seed (70 % of seed weight adhered).

Calomel at one-half weight of seed (48 % of seed weight adhered).

50 % thiram dust at same weight of seed (95 % of seed weight adhered).

The amounts of dust adhering were found by checking weights after treatment. It was necessary with the equal weights of calomel and thiram to re-treat the dried seed with additional sticker to take up the dust which did not adhere on the first treatment. There were four replicates of each treatment. The seed was sown at 28 lb. per acre, but the rows were at right angles to the 1949 rows to avoid error due to previous treatments leaving varying amounts of disease in the soil. The seed was sown on 12 August and the slight check in emergence and early growth was again noted particularly with the equal and 70% rate of calomel. In September there was a severe attack of damping off of the seedlings, again mainly associated with *Corticium (Rhizoctonia) solani*, though *Pythium* and *Phytophthora* spp. were also present on some seedlings. Counts of surviving seedlings were made on 15 October. As can be seen from Table 4, all treatments gave a significantly greater number of plants than the control, though the thiram treatment was inferior to all the calomel treatments. No white rot appeared until there was warmer, dry weather in late April. In order to obtain maximum development of the disease lifting was delayed until 26 June. Seed coats could still be found with calomel adhering to them. All plants were examined for white rot and weights of healthy plants recorded. The results are summarized in Table 4.

TABLE 4. *Effect of seed treatments on stand and weight of onions, variety White Lisbon*

	Control	Seed treatments				S.E.
		100% by wt. calomel	70% by wt. calomel	48% by wt. calomel	95% by wt. 50% thiram dust	
No. of seedlings per yard drill, 15 October	45.6	105.9**	94.8**	93.6**	64.2*	4.61
No. of healthy seedlings per yard drill, 26 June	5.9	72.0**	43.6**	59.7**	10.5	3.76
No. of white rot infected seedlings per yard drill, 26 June	8.9	6.3	13.3	12.0	12.6	1.99
Weight of healthy plants. Tons per acre	1.3	7.5**	5.1**	6.3**	1.7	0.49

* Significant 5% point.

** Significant 1% point.

All the calomel treatments gave a significantly greater number of healthy plants than the control. There are no significant differences in the numbers of infected plants due to the fact that lifting was so long delayed that many of the infected plants in the control rows had rotted completely. The thiram dust gave no control of white rot. The differences in crop weights are highly significant. The comparatively low yields are due to withholding nitrogen dressings in order to keep the experiment going as long as possible and to loss of weight through plants bolting.

Experiments with bulb onions

In 1947 three small trials were laid down with bulb onions in a market garden at Bromsgrove with a history of many crop failures due to white rot. In trial A the variety was Up-to-date and the treatments were: (1) control, (2) soil treated with 4% calomel dust at 2 cwt. per acre, (3) seed treated with 100% by weight of pure calomel, (4) treated seed sown on treated soil. In trial B surplus-treated and untreated seed was sown in an area where onions had repeatedly failed. In trial C the variety was Ailsa Craig and the treatments were: (1) control, (2) 4% calomel dust applied in drill before sowing at the rate of 1 lb. per 50 yd. of drill, (3) seed treated with 100% by weight of pure calomel. All seed was sown by hand on 1 March at a rate of approximately 8 lb. per acre. Emergence was good throughout, but the calomel-treated seed produced plants which were temporarily retarded. White rot was first noted on 18 July. At lifting counts were made of numbers of healthy and infected bulbs in four random samples of 100 bulbs from each plot. The results are given in Table 5. Both seed and soil treatments gave a satisfactory control of the disease, and when both methods were used there was no statistical significance between them.

TABLE 5. *Percentage of onion bulbs infected with white rot following various treatments*

Trial	Variety	Control	Soil treatment	Seed treatment	Seed and soil treatment	S.E.
A	Up-to-date	7	2*	0**	0	1.98
B	Up-to-date	14.8	—	3.2**	—	3.3
C	Ailsa Craig	25.0	1.0**	0**	—	2.4

* Significant at 5% point.

** Significant at 1% point.

DISCUSSION

The observations made in the course of these experiments and also in advisory work in Worcestershire indicate that there are serious gaps in our knowledge of the epidemiology of the white-rot disease. Although ground is known to be so heavily contaminated with the disease that a crop has been completely destroyed it does not follow that subsequent crops will be attacked though the danger persists indefinitely. In our experience the disease tends to become epidemic in onions when there is a check to the growth of the crop. An autumn attack usually occurs if a cool spell follows warm moist conditions. If the cool spell is accompanied by drought the attack tends to be more devastating. The disease ceases to spread when there is a further drop in temperature accompanied by frosts. In the spring an attack invariably follows dry soil conditions. Here again the damage appears to be worse if there is a sudden change from conditions favouring rapid growth of the crop. These observations are in general agreement with the experimental results

in U.S.A. of Walker (1926) that the disease develops most rapidly when at 10–20° and on a fairly dry soil (40% saturation capacity). More information is however required on the effect of fluctuating conditions in the field and the length of duration of appropriate conditions required to induce an attack. On theoretical grounds irrigation in the spring should check the spread of the disease, and experiments were designed to investigate this point but were invariably followed by rain and so failed to produce any results. The presence of the diseases on some sites, especially those carrying their first known susceptible crop, and its absence on adjacent sites of the same soil type under similar conditions also calls for further investigation.

The experimental results obtained over several seasons show that on the salad onion crop seed treatment has provided a good control of the disease. Complete control has not been obtained, but it has been possible to produce a satisfactory commercial crop even if harvesting was delayed until nearly 11 months after sowing in heavily contaminated soil. During the seasons 1949, 1950, 1951 a considerable quantity of seed was treated for or by growers themselves usually at a rate of $\frac{2}{3}$ lb. of pure calomel to 1 lb. of seed using the resin alcohol sticker. A number of these crops were examined, and in every case except one either there was no white rot or the amount was negligible. In one case on heavy wet clay, 25% of the crop was attacked. Unfortunately there was no untreated seed for comparison, but it was impossible to find any calomel on the seed-coats present in the soil. This was the first attempt by the grower to use this treatment, and as in seed treated by ourselves calomel was always found on seed-coats up to 11 months after sowing it suggests that either the correct amount of calomel was not stuck satisfactorily to the seed or, because of the wet cold conditions, it decomposed more quickly than usual. The experiments of Booer (1944) favour this latter hypothesis, but against it in similar conditions during the same season an excellent control was obtained by other growers. During the same period three fields only were seen treated with 4% calomel dust broadcast at $2\frac{1}{2}$ –3 cwt. per acre. In none of these was a control obtained, the amount of disease being 50, 62 and 78%. The incomplete results obtained in field trial 3 also suggest that the soil treatment is inadequate. This is not surprising as 3 cwt. of 4% dust per acre is equivalent to only 13.4 lb. of pure calomel per acre broadcast over the whole area while seed treatment at $\frac{2}{3}$ lb. pure calomel to 1 lb. of seed at the seeding rate of 30 lb. per acre gives 20 lb. of pure calomel in closest proximity to the plants.

With bulb onions the three small experiments gave promising results but they apply only to one site in one season. Under more testing conditions, especially with an autumn-sown crop, it might be necessary to increase the dosage in order to compensate for the much lower seeding rate. By twice treating seed it is easily possible to double the load of calomel on the seed.

For the commercial grower seed treatment has a number of advantages. Once the seed is treated he can sow his crop in the normal way. The results of the 1949

and 1950 experiments show that, in addition to control of white rot, a substantial measure of control of damping off of seedlings is obtained, thus allowing a reduced seeding rate. To use 4% calomel dust, on the other hand, would mean either designing a special combine drill or broadcasting the dust, and the available evidence indicates that this alternative is unsatisfactory. In 1949 the cost of the treatment if carried out by a seed merchant or growers co-operative association was about 7s. 6d. per lb. of seed, approximately £10 per acre using 30 lb. of seed per acre. Unfortunately, the recent rapid increase in price of mercury compounds has trebled the cost in 1952. In spite of this some growers still regard the treatment as economic. It seems, however, that other fungicides must be tested to obtain a satisfactory cheap material. To be satisfactory a compound must not only protect the onion plant from infection for a period up to 10 months but it must not damage the seed. It must also be possible to stick sufficient quantity to the seed without interfering with drilling.

We gratefully acknowledge the co-operation of many growers in making these observations and experiments, and in particular Messrs A. S. Stephens of Norton, Aldridge and Grove of Evesham, and Quarrell of Birlingham. Our thanks are also due to Miss C. F. Ganderton who assisted in making counts in 1950 and 1951.

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THE CONTROL OF ONION SMUT (*UROCYCTIS CEPULAE*) BY SEED TREATMENT

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In field trials near Evesham, Worcestershire, a good control of onion smut was obtained by seed treatment with ferbam and 50 % thiram dusts applied with a resin-potash sticker. A pentachloronitrobenzene compound also gave some control of the disease but reduced emergence. Greenhouse trials confirmed these results, but there was a fall off in control at high levels of infection. Chloranil and calomel were relatively ineffective and two organo mercury compounds caused serious seed injury.

INTRODUCTION

The first published account of onion smut in Britain appeared in 1919 (Cotton, 1919), but apparently it was found earlier than this by W. G. Smith in Scotland in 1912, and by W. B. Mercer in Northumberland in 1914. Attempts to control the disease were made by Mercer and continued by Whitehead (1921), who found that formaldehyde applied in the open drill gave an effective control, but this treatment was never used to any extent as the onion smut Order of 1921 prohibited the planting of onions and leeks in soil contaminated by this fungus. In 1936, by special permission, Ogilvie & Hickman (1937) were able to carry out a trial to test the effects of cuprous oxide and organo mercury compounds as seed treatments against this disease. None of the treatments gave any effective control. Later, in 1942-3 (Moore, 1943, 1948) the Ministry of Agriculture's Inspectors carried out surveys for the presence of onion smut and established that the disease was much more widespread than had been supposed, but seldom caused serious damage. Consequently, the onion smut Order was amended in 1943, and, as it is now no longer illegal to plant susceptible crops on land where onion smut has been known to occur, knowledge of control measures has once more assumed some importance. In 1944, one of us (C.J.H.), while Advisory Officer in Evesham, found a severe case of onion smut in salad onions. With the co-operation of the grower concerned, advantage was taken of this opportunity to try out the seed treatments which Newhall (1943) had found to be effective against onion smut in the United States.

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MATERIALS AND METHODS

Prof. Newhall kindly supplied us with details of the materials and methods which he had used. Following this information, the fungicides first tried were a dust containing 50% tetramethylthiuram-disulphide (thiram), ferric dimethyldithiocarbamate (ferbam), and tetrachlorobenzoquinone (chloranil). In later trials, particularly under greenhouse conditions, the range of substances tested was extended to include compounds containing pentachloronitrobenzene and tetrachloronitrobenzene, three organo-mercury dusts, calomel, 1% diphenyl and copper dimethyl dithiocarbamate (cupram). Unless otherwise stated, the weight of dust used was equal to the weight of seed treated. In our first experiment we used a sticker recommended by Prof. Newhall in order to obtain adherence of the fungicides to the onion seed. This sticker was prepared by dissolving 14.25 g. of caustic potash in 250 c.c. of water. This was heated and to it was added 57 g. of powdered resin. The solution was boiled until clear and after cooling diluted to give a Baumé gravity of 5. This sticker was used at the rate of 1 c.c. to 5 g. of seed. The seed was shaken for about 2 min. with the sticker to secure even dispersal, the appropriate amount of fungicide was then added and shaking continued until it all adhered to the seed which was then spread out in a thin layer to dry. In later experiments water-paste sticker, as recommended by Wright (1939) for use in applying calomel to onion seed for the control of onion fly, was used. It was thought that this material might easily be obtained by growers, but not only did it tend to give less even distribution of the fungicide but also reduced the tenacity of the coating. A synthetic resin dissolved in alcohol supplied by Dr H. Martin was also tried. This proved even more effective than the resin potash sticker and it was possible to reduce the quantity of sticker to 1 c.c. to 20 g. of seed and still retain the same weight of fungicide on the seed. This sticker also had the advantage that it was possible to dry the seed very quickly.

FIELD TRIALS

The first field trial was laid down at Lenchwick, near Evesham in 1944. The land had carried a severely infected salad onion crop in the autumn of 1943. It was dug in late February, raked, rolled and sown by hand on 6-7 March. There were four treatments: control, ferbam, 50% thiram and chloranil, each of the dusts being applied with resin-potash sticker. Five replicates of each treatment were arranged in randomized blocks, each replicate containing 50 yd. of drill. Each plot was sown with the same quantity of seed, variety 'Bedfordshire Champion', the seed rate being approximately $7\frac{1}{2}$ lb. per acre. The seedlings were examined when they were about 2 in. tall. In each plot five 2 yd. lengths of row were selected at random. The number of healthy seedlings was counted and also the number infected by smut. In July further counts were made in the same manner. By this time most of the

infected seedlings had died but there was little change in the population of healthy plants. The results are summarized in Table 1.

TABLE 1. *Numbers of healthy and smut infected onion seedlings, variety 'Bedfordshire Champion', per foot of drill*

	Control	Ferbam	50 % thiram	Chloranil	S.E.
Healthy plants, 27 Apr. 1944	2.4	5.6**	4.9	3.3	0.39
Infected plants, 27 Apr. 1944	2.08	0.36	0.62**	0.79	0.106
Healthy plants, 13 July 1944	2.1	5.4**	4.2**	3.4	0.55
Infected plants, 13 July 1944	0.14	0.02	0.03	0.03	0.013

** Difference statistically significant from control at 1 % point.

Both the ferbam and 50 % thiram treatments gave a population of healthy plants significantly greater at the 1 % point than that of the untreated rows. That this is due to control of onion smut is shown by the correspondingly fewer infected plants in these treatments. Chloranil reduced the number of infected plants significantly, but the number of healthy plants surviving just failed to be statistically significantly greater than in the controls, indicating that the treatment might have been phytotoxic.

The site was cleared in August, the ground re-prepared and a second trial sown on 8 September. The treatments used were ferbam, thiram, 4 % calomel dust, a dust containing pentachloronitrobenzene, all applied as in the previous experiment. In addition the thiram treatment was repeated but using the water paste sticker instead of the resin potash sticker. The variety of onion was 'Ebenezer' and the seeding rate approximately $7\frac{1}{2}$ lb. per acre. Counting infected plants in the field had proved difficult in the first trial and greenhouse experiments had shown that in some plants lesions occurred below soil level. Therefore on 29 November the plants were lifted from ten random samples of 2 ft. drill from each plot and examined in the laboratory. Unfortunately by this date some of the infected plants had died and disappeared. The results are given in Table 2.

TABLE 2. *Numbers of healthy and smut-infected onion seedlings, variety 'Ebenezer', per foot of drill*

	Control	Ferbam	50 % thiram	50 % thiram (water paste sticker)	Pentachloro- nitrobenzene	4 % calomel	S.E.
Healthy	2.85	6.0*	5.7*	3.8	5.0	2.7	0.90
Infected	0.45	0.05*	0.06*	0.10*	0.05*	0.45	0.0121

* Difference significant from control at 5 % point.

The plant populations were slightly higher than those in the previous experiment and both the ferbam and thiram treatments again gave stands double that of the controls. The substitution of potash resin sticker by water paste gave a poorer stand of healthy plants and more smut-infected ones. This is attributed to the fact that although the same amount of dust adhered to the seed originally it did not stick so well and some was lost before sowing. The tetrachlorobenzene compound gave the same number of smut-infected plants as the ferbam and thiram treatments but a lower number of healthy plants.

GREENHOUSE TRIALS

Concurrently with and continuing after the field trials a number of experiments were made in a greenhouse to compare with the results of the field trials and also to test a wider range of chemicals. In each experiment 200 seeds were sown just below the soil surface in seed trays 2 in. deep, and there were four replicates of each treatment and control.

Experiment 1

For this experiment contaminated soil from the Evesham trial site was used. The treatments were ferbam, thiram, a pentachloronitrobenzene compound, and 4% calomel dust, all applied with resin potash sticker and also thiram applied with water paste. The seed was sown 31 August 1944, and the seedlings removed and examined on 22 September when they were about 2 in. high. The results are given in Table 3.

TABLE 3. *Mean numbers per seed tray of healthy and smut-infected onion seedlings, variety 'Bedfordshire Champion'*

	Control	Ferbam	50 % thiram	50 % thiram (water paste)	Pentachloro- nitrobenzene	4 % calomel	S.E.
Healthy	62	111**	111**	114**	49*	93**	3.1
Infected	29.6	1.0**	0.6**	0.4**	6.6**	15.8**	1.62

* Difference significant at 5 % point.

** Difference significant at 1 % point.

As in the field, the ferbam and thiram treatments gave almost double the number of healthy plants appearing in the control, and less than 1% of the plants from treated seed were infected compared with 32% of the control plants. The substitution of water paste for resin potash sticker did not affect the efficiency of thiram but there was less opportunity to lose dust than in the field experiment, as the seed was sown immediately after treatment. The pentachloronitrobenzene preparation reduced the number of infected plants but caused a significant decrease in numbers of healthy seedlings due to reduced emergence. The calomel treatment gave an increased stand of healthy and less infected plants than the control but was much inferior to ferbam or thiram.

Experiment 2

The soil used in Exp. 1 was mixed together and the trays refilled and the experiment repeated. The seed was sown on 28 September and the plants removed and examined on 30 October. These results are summarized in Table 4. Resowing in contaminated soil provided a more severe test of the treatment. The ferbam and thiram treatments still gave many more healthy and fewer infected plants than the control, but there was a very marked rise in the number of infected plants from treated seed.

TABLE 4. *Mean numbers per seed tray of healthy and smut-infected onion seedlings, variety 'Bedfordshire Champion'*

	Control	Ferbam	50 % thiram	50 % thiram (water paste sticker)	Pentachloro- nitrobenzene	4 % calomel	S.E.
Healthy	10	63**	44**	50**	14	25	5.3
Infected	80	50*	67	60*	40**	79	6.52

* Difference significant from control at 5 % point.

** Difference significant from control at 1 % point.

Experiment 3

The soil used in Exp. 2 was mixed with an equal quantity of sterile compost thus enabling more treatments to be tried. Ferbam was not available and was replaced by the corresponding copper compound, cupram. The 4 % calomel dust was replaced by pure calomel and there were also included two organo-mercury preparations. As the pentachloronitrobenzene compound appeared to be phytocidal a preparation containing trichloronitrobenzene was included to see if this would prove less harmful. In addition to thiram applied with resin potash a resin alcohol material was also used as sticker. A preparation containing 1 % diphenyl was also included. The seed, variety 'Ebenezer', was sown on 4 December and seedlings examined on 29 January. The results are shown in Table 5.

Dilution of the contaminated soil with an equal quantity of compost appeared to have brought the potential infection back to about the same level as in the first greenhouse experiment. Both thiram treatments and the pure calomel treatment produced numbers of healthy seedlings greater than, and infected seedlings less than, those of the controls. The differences were significant at the 1 % point. In this experiment the pentachloronitrobenzene preparation had less adverse effect on germination and gave the same number of healthy seedlings as cupram, significantly greater than the control at the 5 % point. The two organo mercury preparations and the trichloronitrobenzene compound seriously reduced germination. The 1 % diphenyl preparation had no influence on germination or on smut control.

TABLE 5. *Mean numbers of healthy and smut-infected onion seedlings, variety 'Ebenezer'*

	Healthy	Infected
Control	72	38
Cupram (resin potash sticker)	106*	2.5**
Thiram (no sticker)	134**	1.0**
50 % thiram (resin alcohol sticker)	169**	0.5**
Pure calomel (resin potash sticker)	141**	0.5**
Organo-mercury A (resin potash sticker)	29	15*
Organo-mercury B (resin potash sticker)	1**	12**
Pentachloronitrobenzene (resin potash sticker)	105*	1.5**
Trichloronitrobenzene (resin potash sticker)	12**	2**
1 % diphenyl (resin potash sticker)	88	35
S.E.	16.8	5.2

* Difference significant from control at 5 % point.

** Difference significant from control at 1 % point.

Experiment 4

The soil used in Exp. 3 was thoroughly mixed and the trays refilled. They were then resown on 14 March using the following treatments: 50% thiram dust, pentachloronitrobenzene, pure calomel, organo-mercury A, organo-mercury B, all applied with resin potash sticker. The amounts of organo-mercury A and B were reduced to 50% by weight of seed to try to reduce injury. In addition, 50% thiram dust with resin alcohol sticker, and pure thiram without sticker were also included. The seedlings were removed and examined on 11 April. The results are summarized in Table 6.

TABLE 6. *Mean numbers of healthy and smut-infected onion seedlings, variety 'Ebenezer'*

	Healthy	Infected
Control	38	73
50 % thiram (resin potash sticker)	65*	33**
50 % thiram (resin alcohol sticker)	71**	39**
Pure thiram (no sticker)	89**	45**
Pure calomel (resin potash sticker)	54	65
Pentachloronitrobenzene (resin potash sticker)	83**	28**
Organo-mercury A (resin potash sticker)	50	61
Organo-mercury B (resin potash sticker)	19	5**
S.E.	8.25	4.60

* Difference significant from control at 5 % point.

** Difference significant from control at 1 % point.

Again the level of infection had risen appreciably but the pure thiram, 50% thiram with resin alcohol sticker, and the pentachloronitrobenzene compound gave approximately twice as many healthy plants and half as many infected plants as in the controls. The 50% thiram with resin potash sticker was slightly less effective. None of the mercury compounds gave any significant control of the disease though the amount of damage done by organo-mercury A was reduced.

DISCUSSION

In the two field trials ferbam and 50% thiram dusts applied with a resin potash sticker gave a satisfactory control of onion smut. The pentachloronitrobenzene compound used in one trial reduced the amount of smut present but had apparently a phytotoxic effect. This effect was confirmed in greenhouse trials—the compound reducing the number of plants emerging on two occasions, though always giving some control of smut. This behaviour appears to rule it out for field use unless the damage it causes can be prevented. Although the resin alcohol sticker was more efficient than the resin potash sticker it was not readily available at the time of these experiments. Later, however, a similar cheap material was obtained and proved equally effective. It was used for experiments on control of white rot by seed treatment (Croxall, Sidwell & Jenkins, 1953).

In the greenhouse experiments ferbam and 50% thiram were again the most consistently successful of the compounds used, although they were less effective at high levels of infection. Similar results have been noted by American workers. Nelson (1946) found that dusts were as effective as formaldehyde treatment when infection was moderate but not so good when the attack was severe. Linn & Newhall (1948) obtained results which also illustrated the falling off in control when high levels of infection were encountered. However, in this country so far (Moore, 1948) losses above 30% have not been reported and in our field trials a good control was obtained even when a second onion crop was grown on contaminated land.

If other land is available onions and leeks should not be grown on contaminated land. However, on many small holdings in the Vale of Evesham salad onions are an important crop and land suitable for their production is very limited. It was on such holdings that many of the cases of onion smut were recorded in the 1942–3 surveys (Moore, 1948). In the salad onion crop the damage caused by smut may be underestimated as onions may be attacked before emergence and lesions above ground are not easily seen in a thick stand. Moreover, as seeding rates are high the grower does not become alarmed unless a substantial percentage of seedlings are killed. Seed treatment with ferbam or 50% thiram dusts offers a simple means of preventing the disease building up on land frequently cropped with onions. Unlike the formaldehyde treatment no special apparatus is required and rain immediately after sowing is not likely to affect adversely the control obtained. The seed treatment is quickly carried out and the treated seed passes readily through the seed drill though the aperture should be increased slightly to allow for the bigger size of the treated seed. If onion white rot, caused by *Sclerotium cepivorum*, is also present in the soil the possibility of combining the seed treatments against both diseases might be considered.

The authors wish to express their thanks to Mr L. Ogilvie for his interest and help during the course of this work, and to Miss B. Smith who assisted in making some of the infection counts.

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BREEDING PEARS FOR RESISTANCE TO THE PEAR SCAB FUNGUS *VENTURIA PIRINA* ADERH.

I. VARIATION IN THE PATHOGENICITY OF *VENTURIA PIRINA*

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(With 2 Text-figures)

Three clones of *Venturia pirina* have been tested for their pathogenicity towards forty varieties of cultivated pear. From observations on the reactions of these forty varieties, twelve were selected for differentiating the pathogenicity of sixteen clones of the fungus.

These tests differentiated four main groups of biotypes of *V. pirina*. Clones isolated from Conference showed wide differences in range of pathogenicity. One isolate from Conference was shown to parasitize six of the most widely grown pears in England: other Conference biotypes had a narrow range.

No variety of pear was found immune to all the isolates. Beurré Giffard was lightly infected by an isolate from Durondeau, but was resistant to all the other isolates tested.

INTRODUCTION

A previous paper (Stanton, 1952) describes the variation in culture of isolates of the pear scab fungus *Venturia pirina* and the relationship between this variation and that of the host. This paper considers the variation in pathogenicity of a number of the isolates, collected for the previous study, when inoculated on to a group of host varieties.

Studies on biotype specialization in plant pathogenic fungi have been numerous, especially among the Uredinales. Noteworthy among these researches are those of de Bary (1879), Eriksson & Henning (1896), Stakman & Levine (1922). Further, biotype specialization has been recorded in practically the whole range of plant parasites (Imms, 1931). Reviews of the subject for insects have been given in greater detail by Thorpe (1930) and also by Mayr (1947).

It was to be expected, therefore, that specialization should occur in the species of *Venturia*. This was demonstrated for *V. inaequalis* by Wiesmann (1931) and was confirmed for *V. pirina* by Herbst (1936). Research on variability in pathogenicity has been extended in these fungi by Palmiter (1932, 1934), Rudloff (1934), Schmidt (1935), Kuthe (1935). The segregation of the pathogenicity factors responsible for biotype formation in *V. inaequalis* was first demonstrated by Keitt, Palmiter & Langford (1938) and fully reported by Keitt & Palmiter (1938) and for *V. pirina* by Langford & Keitt (1942).

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The present account is concerned with the behaviour of conidial isolates. In particular the variability of forms found on the pear variety Conference has been studied. The preponderance of Conference in pear growing in England is largely due to its scab resistance.

TABLE 1. *Classification of pear varieties according to their field susceptibility to scab*

	Origin	
	Year	Place
Immune		
Beurré Giffard	1825	France
Very resistant		
Dr Jules Guyot	1870	France
Conference	1894	England
Beurré Mortillet	1875	France
Resistant		
Beurré Hardy	1820	France
Bristol Cross	1930	England
Moderately resistant		
Durondeau	1811	France
Fondante de Thirriott	1858	France
Doyenné du Comice	1849	France
Susceptible		
William's Bon Chrétien	1770	England
Fertility	1875	England
Glou Morceau before	1800	France
Winter Nelis	1818	France
Belle Julie	1832	France
Laxton's Superb	1920	England

Table 1 shows little connexion between the age of the variety and its present field susceptibility. For instance, Beurré Giffard, now 126 years old, ranks highest for resistance and is closely followed by the 80-year-old variety Dr Jules Guyot. Among the susceptible groups are four very old varieties and the resistance factors present in these old varieties are likely to be of particular value in breeding work.

MATERIALS

Pathogen

Isolation, morphological and physiological variation of the pathogen have been described in a previous paper (Stanton, 1952). Selected isolates found during this study have been used here.

Host

The growth of many pear varieties is found to be unsatisfactory on quince rootstocks in pots, even when the variety is double worked. Therefore, varieties were worked on to seedling pear. The seedlings, raised from open-pollinated Conference pear, are selected for median vigour before planting out in May for

budding at the end of July. The trees are lifted in the October of the succeeding year and potted in a standard medium. A comparison between trees grafted in this way and trees grafted on quince A showed no significant difference in severity of symptoms to pathogenic clones of the fungus. The potted trees are plunged in open pits filled with screened ash until required.

Host material of the above type was used in 1950 and 1951. Trees for inoculation were brought into the greenhouse in January and kept at 20° C. day temperature and 18° C. night. When 2 mm. of green tissue was showing between the bud scales, approximately 3 weeks from the time the trees were brought in, they were cut back to within 15 cm. of the graft union and four buds allowed to develop.

Shoots were inoculated when 25 cm. long, and labelled on the petiole of the youngest expanding leaf. This label indicates the stage of development at inoculation, since five or more leaves may develop between inoculation and scoring. The labelled leaf is designated L^0 , leaves above being designated L^{+1} , etc. and below L^{-1} , etc.

INOCULATION

Spore suspensions were taken from 14-day-old cultures kept at 18° C. on a yeast-dextrose agar slope. This suspension was used to inoculate tubes containing liquid yeast-dextrose media on a strip of filter pad. Single-spore cultures were made at the same time as a check against morphological and physiological variation. After a further 14 days the conidia were applied to the host, using the polythene sleeves previously described (Stanton, 1951).

The inoculum was prepared by shaking the culture tube, filled with distilled water, gently for 1 min. and then pouring the spore suspension through muslin (McCallan & Wilcoxon, 1939). The suspension was then adjusted to contain, approximately, 1000 spores per ml. Many clones of *V. pirina* sporulate very poorly in culture, but no difficulty was encountered in obtaining 20 ml. or more of a suspension of this strength. 1 ml. of inoculum, approximately, was used per shoot (leaf area 100–200 sq.cm.) giving a theoretical mean density of spores between 5 and 10 per sq.cm. In practice it was found that densities of the order of between 0.2 and 1.0 sporulating lesions per square centimetre were obtained.

The inoculum was sprayed on both surfaces with a long-stemmed glass nasal atomizer. The sleeve was kept closed for 3 days, after which time it was assumed that 95 % of the spores had germinated sufficiently to penetrate the leaf (Viennot-Bourgin, 1946). The sleeves were then removed completely.

Greenhouse conditions

The trees were incubated in a greenhouse cubicle provided with top and side ventilation and a forced draught. From May until September, when inoculations ceased, it was found necessary to screen with permanent green shading on the south and west sides of the cubicle.

Incubation lasted for 6 weeks, the trees being scored for infection at 2, 4 and 6 weeks from inoculation.

Temperature was maintained as far as possible at 18° C. with a lower limit of 15° C. and an upper of 21° C. Syringing of the trees was frequent, varying from 3 to 10 times per day according to sun intensity.

Pest and disease control

Effective sucking insect control was achieved using the proprietary phosphorus insecticide E.P.N. 300 (*O*-ethyl-*O*-*p*-nitrophenyl benzenethionphosphate). Mildew (*Podosphaera leucotricha*) was controlled by frequent overhead spraying (2–5 times daily) with rainwater.

Host selection and method of scoring results

The experiments described below were made, not to differentiate the products of ascus segregation (Keitt *et al.* 1938), but to explore the variation in pathogenicity occurring in field populations of the fungus. That is, it was required to estimate biotype differentiation in the whole population and more critically in the Conference isolates. Further knowledge was required of how the different biotypes affected the field population of pear varieties from 'field susceptible' to 'field resistant'.

Experiment 1

A preliminary series of inoculations using three clones of *Venturia pirina* which differed in host-origin, was made on to a range of varieties. The clones used are single conidial isolates from Glou Morceau 29³, Conference 51⁶ and Durondeau 52⁴. The characteristics of these clones are described in detail elsewhere (Stanton, 1953). From field behaviour, these varieties were known to span the range from field-resistant to field-susceptible to scab.

The results of these infections, made between March and June 1950, are given in Fig. 1. The symbols for Figs. 1 and 2 are set out below. A *four-type* scoring, according to the degree of attack, has been used. This shows more clearly the reaction of hosts for these studies, than the two-type classification of Keitt.

Type O. No visible mark or lesion on the host at the end of 1 month after inoculation.

Type +. A chlorotic spot varying in size from 0.5 to 2.5 mm. in diameter, but without a necrotic centre, at the end of 1 month.

Type ++. A chlorotic spot at a fortnight after inoculation, which develops at 1 month into a necrotic spot, up to 2.5 mm. in diameter, surrounded by a chlorotic halo. This type of lesion bears a few spores at the edge of the necrotic area.

Type +++. A sporulating lesion which develops between a fortnight and 1 month after infection, usually without any necrosis. Necrosis may set in later and extend to 5 mm. or more diameter at the end of 6 weeks. The lesion is often found to be very diffuse and this phenomenon is associated with the 'sparse' character found in culture.

At first sight these results seemed confusing, in that clones of the organism, isolated from a field-resistant variety, Conference (clone 51⁶), did not always produce an infection on the field-susceptible varieties. The second feature of these



Fig. 1.

results was the greater percentage of infections produced by the clones isolated from the two field-susceptible varieties, Glou Morceau (clone 29³) and Durondeau (clone 52⁴). 51⁶ infected nine varieties, 29³ infected twenty-one and 52⁴ infected twenty-three. None of the varieties showed complete immunity to all three clones. For the next experiment, twelve of the varieties used in Exp. 1 were selected to

confirm the differentiation between clones 51⁶, 29³ and 52⁴ and to try other related clones of the fungus for further differentiation.

Experiment 2

The varieties, sources of clones and the results of inoculations are given in Fig. 2. These particular varieties were chosen for clarity of reaction to the fungus and then for one or more of the following reasons:

(a) Ability to differentiate clones. (Chosen on these grounds were: Beurré Giffard, Dr Jules Guyot, Beurré Mortillet, Doyenné du Comice, Belle Julie, Winter Nelis.)

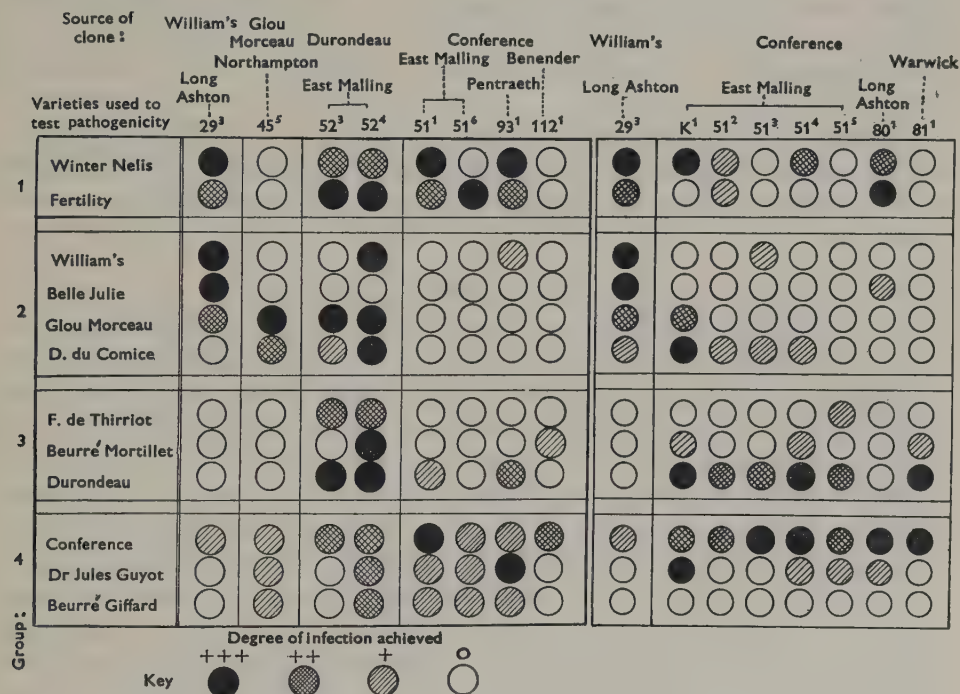


Fig. 2.

(b) Being the original host varieties from which the isolates were made.

(c) Being widely grown varieties (Conference, Durondeau, Doyenné du Comice, William's, Fertility).

Each infection result is deduced from four shoot inoculations. (Four trees of each variety were used and four inoculations made per tree.) No tree was twice inoculated with the same clone. Inoculations were made in July 1950 and repeated in March 1951 (Exp. 2a). Using another set of forty-eight trees, a further eight geographically differentiated clones, from Conference, were tested in March 1951 and again in July 1951 (Exp. 2b).

The results of this experiment confirmed the earlier work and Fig. 2 has been subdivided to show how further differentiation has appeared, varieties with similar reaction being placed in the same group.

Thus it appears that Winter Nelis and Fertility are similar in their susceptibility to clones from William's, Durondeau, and to eight out of eleven Conference clones. Variety groups 2 and 3, in Fig. 2, are generally not attacked by Conference clones. Group 3 differs from group 2 in that it is not attacked by the Williams and Glou Morceau clones. Group 4 is severely attacked only by the Durondeau and Conference clones. Within this last group there seems to be an increasing order of resistance thus: Conference, Dr Jules Guyot and Beurré Giffard. The variation in isolates taken is partly attributed to the possibility that the clone may not have been isolated from or tested on its most susceptible host variety. The clone K^1 is of particular interest in that it has an exceptionally wide range and that the varieties it attacks are widely cultivated.

In contrast to the demonstrably limited range of certain biotypes, is the field observation that practically all pear varieties are attacked by scab, in some degree, and to reconcile these two observations it is necessary to postulate a large number of biotypes.

DISCUSSION

Clones of *Venturia pirina* show two orders of variation in pathogenicity. First, a major order which divides the clones into groups, and secondly, a minor order of variation within the groups. These *biologic strains* of the fungus, which may be called Glou Morceau strain, Durondeau strain, or Conference strain, each comprise a number of biotypes. It has been shown that strain differentiation is not a step-by-step increase in severity of attack, but rather a number of qualitatively different resistance systems some of which are more easily overcome than others.

Two factors may make the limits of host range of the different *V. pirina* biotypes obscure. First, the host is a complex polyploid, although it shows secondary diploidization (Darlington & Moffat, 1930; Moffat, 1931; Derman, 1949). This situation accounts for the difficulties associated with the genetical analysis of the pome fruits (Crane & Lewis, 1949) and the phenomenon of continuous variation shown by a number of characters. Secondly, the fungus, being a facultative parasite, is not so dependent on a close relationship with the host as an obligate parasite and its growth is not, therefore, so likely to be terminated abruptly by an unfavourable host as in the immune and hypersensitive reactions of obligate parasites.

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BREEDING PEARS FOR RESISTANCE TO THE PEAR SCAB FUNGUS, *VENTURIA PIRINA* ADERH.

II. THE STUDY OF FIELD RESISTANCE ON SELECTED PEAR SEEDLINGS AND THE INHERITANCE OF RESISTANCE IN SEEDLING PEAR FAMILIES UNDER CONTROLLED CONDITIONS

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(With 1 Text-figure)

Clonal isolates of *Venturia pirina*, shown in a previous paper to differ in range of pathogenicity, have been used to inoculate the following pear seedlings: (i) resistant seedlings from families which appeared to be segregating for resistance under field conditions, (ii) two whole families of field-resistant \times field-susceptible parentage.

In (i), it was found that clones of the fungus could infect some of the seedlings hitherto regarded as field-resistant. In (ii), segregation for susceptibility in the two families varied, according to the clone of the fungus used, from approximately a 1:1 ratio down to 'all resistant'. Resistance to individual clones appeared to be dominant to susceptibility, but further data are required before attempting a complete interpretation.

There was a general correlation between susceptibility to the different clones. Those that produced the most infection were isolated from the parental varieties of the seedlings. This correlation was not, however, complete and clones differing in host origin tended to attack different groups of seedlings.

INTRODUCTION

It has been established by a number of workers (Wiltshire, 1915; Palmiter, 1932, 1934; Marsh, 1933; Schmidt, 1937; Keitt & Palmiter, 1938) that varietal resistance to *Venturia* species as a physiologic property of the host, exists in cultivated varieties of apples and pears. It is concluded that, for the apple, complete immunity does not occur among either European or American varieties, though a greater degree of resistance and possible immunity may occur in the pear. More recently, it has been demonstrated that complete immunity may be obtained in some *Malus* species (Shay & Hough, 1948; Hough & Shay, 1949) and that these species may be crossed with cultivated varieties. Further, it appears that some general resistance to scab may be simply inherited (Shay & Hough, 1948), although the statement must be regarded with reservation in view of the variation in the parasite: if this were so, it would greatly simplify selection in the intermediate generations between the F_1 and the final generation. However, the observations of Crane & Lewis (1949), on the field susceptibility to scab of a number of seedling families of cultivated varieties of pear,

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demonstrate that a high degree of field resistance may be obtained in crosses of cultivated varieties. They further indicate the relative value of different parents in contributing to the resistance of the progeny. Inheritance of scab resistance they regard as complex. The complexity of their results is understandable in that the observations were made on an epidemic attack. Variation in intensity of infection is due to infection spread from a series of foci, variation in the fungus, climatic variations and variations in the vigour and constitution of the trees due to soil, stock and inherent variation (these last-named factors have been shown to cause a large variation in the predisposition of a seedling to a clone of the pathogen).

Selection for resistance to scab is possible early in a breeding programme. This paper considers the reaction of pathogenic clones of the fungus to field-selected seedlings and seedling families and demonstrates the number of biotypes of the fungus required in the rigorous selection for scab resistance.

MATERIALS AND METHOD

The characteristics of the clones used in these studies have been described previously (Stanton, 1952, 1953), as have also the artificial infection methods and method of scoring. Two types of material were used. First, that for the study of field-resistant seedlings was obtained by grafting on Conference seedlings scions from the seedlings which were 'scab free' from the Long Sutton observations (Crane & Lewis, 1949, table 9, column 5). Secondly, the seedling families used in these studies were as shown in Table 1.

TABLE 1

Family	I	II
Parentage	Conference \times Fertility	Doyenné du Comice \times Beurré Giffard
No. of seedlings	41	60
Year of origin	1937	1938
Year of budding	1949	
Stock	Quince A	
Lifted and potted	October 1950	

The trees were cut back to within 15 cm. of the graft union in January 1951, and four shoots were allowed to develop. Similarly, four shoots were encouraged to develop on the scions of the Long Sutton seedlings.

RESULTS

(1) *Inoculations on the field-resistant Long Sutton seedlings*

The results in Table 2 are those of four inoculations (2×2 replicates) except where the scoring is followed by a question mark. A range of *V. pirina* clones was used including the commoner types on Glou Morceau and Williams and clones known successfully to parasitize Conference.

It will be seen that three of the eight seedlings (G.F. 2, G.F. 4, G.F. 5) supported fully sporulating lesions for one or more isolates and that these three were all capable of being attacked by the isolate Conference 51⁶. From previous studies, this isolate appears to be one possessing a relatively wide range. Of the other five, two, G.C. 1 and G.F. 6, might be expected to remain free from scab under a wide range of field conditions and this statement holds, with less certainty, for the seedlings G.F. 1, G.F. 3 and G.C. 2.

TABLE 2. *The reaction of field resistant seedling pears to selected clones of Venturia pirina*

Selected seedlings tested		Clones used as inocula					
		From Glou Morceau		From William's	From Durondeau	From Conference	
Parentage	Seedling no.	45 ⁵	47 ⁴	29 ⁸	52 ⁴	51 ⁸	51 ⁶
Beurré Giffard	G.C. 1	o	o	o	o	o	o
× Conference	G.C. 2	++	+	o	o	o	+
Beurré Giffard	G.F. 1	o	o	o	o	o	++
× Fertility	G.F. 2	o	o	o	+++	o	+++
	G.F. 3	o	o	o	o	++	o
	G.F. 4	o	+++	o	o	o	+++
	G.F. 5	o	++	+++	+++	o	+++
	G.F. 6	o	+	o	o	+	o

Key. Degree of infection produced: + + +, freely sporulating lesion; + +, feebly sporulating necrotic spot; +, chlorotic spot; o, nil. For further details of these reactions see Stanton (1952).

(2) *Inoculations on the seedling families*

The experiments, necessarily limited by the available seedling families, were designed to give a preliminary indication of the discreteness of resistance inheritance and to show how demonstrably different clones behaved on whole families. For comparative purposes, they are presented as a figure (Fig. 1), although the actual inoculations were performed at different times.

Some of the clones have been inoculated on to both families, others occur on only one. It appeared early in these inoculations that clones isolated from varieties other than the parental varieties produced few infections on the appropriate seedling family. Thus, neither Conference nor Fertility isolates infected more than one or two seedlings from the Comice × Giffard family.

In Fig. 1 the seedlings have been placed in ascending order of resistance and the two families have been scaled so that they are comparable on a percentage basis.

Each of the small rectangles in the figure represents the different reactions of a particular seedling to one clone. The 'infected' category is divided into high and low to distinguish between those seedlings which were clearly 'fully susceptible' and those which were 'probably fully susceptible'.

Fig. 1 shows that the frequency of infection on the Comice \times Giffard family (2) is generally less than on the Conference \times Fertility family (1). Family 2 is almost completely resistant to all clones other than those originally isolated from Comice. (Unfortunately, no Giffard isolates were available.) This high order of resistance is only shown in family 1 to the clone isolated from Glou Morceau (45^5).

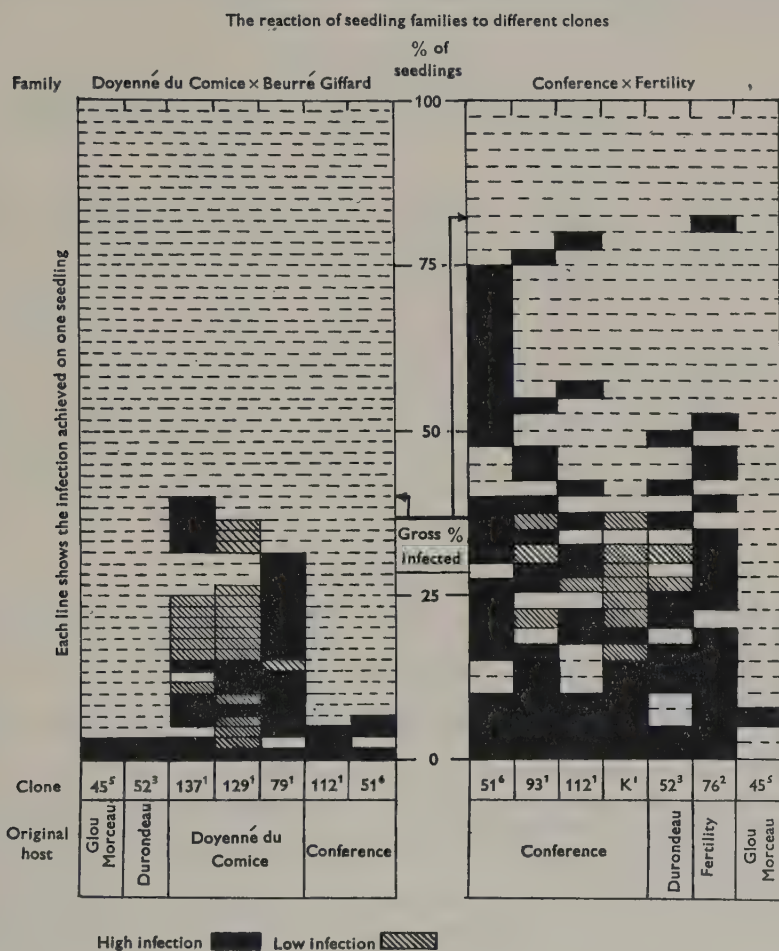


Fig. 1.

It appears that there is a correlation not only between susceptibility to clones from the same variety, but also between clones from different varieties, i.e. that there is a common basis for susceptibility to different clones. The correlation is not complete, however, and in particular there are opposite reactions shown by clones 51^6 and 52^3 just as these clones behave differently on a group of varieties.

Minor differences also occur between the different Conference isolates. This was to be expected in view of their different reactions on the variety groups.

Hough & Shay (1949) report simple ratios of resistant to susceptible in their work, and I have therefore compiled my results in the form of ratios for comparison (Table 3). It will be seen that resistance appears to be dominant to susceptibility to all clones. Further work will be required to establish clearly the ratios involved as in only a few of the results are simple ratios apparent.

TABLE 3

Family ...		(1) Conference \times Fertility			(2) Comice \times Giffard		
Source of clone	Clone no.	No. of seedlings S:R	% R	Ratio S:R	No. of seedlings S:R	% R	Ratio S:R
Conference	51 ¹	10:30	75	1:3.0	—	—	—
	51 ⁶	18:23	56	1:1.27	5:55	92	1:11.0
	93 ¹	15:22	61.5	1:1.47	—	—	—
	K ¹	14:26	65	1:1.85	—	—	—
	112 ¹	6:35	85.5	1:5.85	2:58	96	1:29.0
Comice	79 ¹	—	—	—	18:40	69	1:2.22
	125 ¹	—	—	—	10:37	79	1:3.7
	137 ¹	—	—	—	10:37	79	1:3.7
Durondeau	52 ³	7:34	83	1:4.85	2:58	96	1:29.0
Glou Morceau	45 ⁶	0:41	100	All res.	2:58	96	1:29.0
Fertility	76 ²	16:24	60	1:1.5	—	—	—

S:R=susceptible:resistant. All res.=All resistant.

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OBSERVATIONS ON THE EFFECT OF GROWTH-STIMULATING COMPOUNDS ON THE HEALING OF WOUNDS ON APPLE TREES

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(With 2 Text-figures)

Wounds were made at various times of the year on the trunks and large branches of apple trees with a 1 in. bit and treated with a number of organic compounds in lanoline paste. The healing of these wounds was followed through two growing seasons by tracing and measuring the area of exposed wood at different times after wounding.

Callus growth was practically confined to the summer months. Development of callus was improved by applications of lanoline, and the lanoline effect could be further enhanced in the early part of the first growing season following treatment by the addition of certain growth-stimulating compounds including 4-chloro-3:5-dimethylphenoxyacetic acid and 2:4-dichlorophenoxyacetic acid. Indolyl-3-butyric acid probably also caused some stimulation. After the period of initial stimulation the rate of callusing was approximately the same on treated as on untreated wounds.

INTRODUCTION

The treatment of both natural and artificial wounds is of particular importance in cultivated trees subject to frequent and drastic pruning. While many untreated wounds heal satisfactorily, the provision of protection by using wound dressings containing fungicides or bactericides may be advantageous.

The importance of selecting wound dressings which encourage callus development has been recognized by a number of authors: Marshall (1931) showed that painting with shellac would improve healing, and Shear (1936) attempted to stimulate callus development by treating wounds with lanoline containing indole-acetic acid. Shear found that lanoline alone improved healing and was more effective than lanoline with indole-acetic acid. Jakes & Hexnerová (1939) found that a lanoline paste containing 1 % indole-acetic acid increased the rate of healing of wounds on the terminal shoots of a number of fruit trees. Oliver & Grace (1940) noticed that linseed oil pastes containing indolyl butyric acid caused some early stimulation of bark wounds of young trees of *Fraxinus pennsylvanica lanceolata*. Crowdy (1948) attempted to check the spread of apple cankers (caused by *Nectria galligena* Bres.) by stimulating the natural regeneration of the host. A number of the plant growth-regulating compounds used appeared to stimulate the callusing of

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wounds on apple shoots, so these materials were selected for trial as wound dressings. A preliminary account of these wound-healing trials has already been published (Crowdy, 1949*a*).

EXPERIMENTAL METHODS

The methods employed to study wound healing were in general similar to those described by Marshall (1931). Holes were bored each month in the trunks or larger branches of apple trees with a bit 1 in. in diameter: the depth of the holes was such that the cylinder of tissue removed included the youngest and most active wood cells. In this way observations were confined to callusing at the edges of the wound and the healing at the surface of the wood reported by Sharples & Gunnery (1933) was avoided. The outline of the exposed wood surface in these holes was traced on celluloid sheet and later retraced on to paper to provide a permanent record. The area of exposed wood represented by these tracings was measured with a planimeter. In some cases the peripheral tissues adjacent to the wound split at the time of wounding; wounds seriously affected in this way together with those which later developed infections or were lost in pruning were omitted entirely from the analysis of the results. This procedure unbalanced the design of the experiment and, where the results were subject to a statistical analysis, the balance of the design was restored by omitting comparable but normal wounds in the other treatments. In theory the initial area of exposed wood at wounding should be about 5 sq.cm. but it was found in practice that there was some shrinkage of the surrounding peripheral tissues and the average initial area was more usually in the neighbourhood of 5.5 sq.cm.

The compounds were all applied as lanoline pastes which were kept in lengths of glass tubing $\frac{3}{8}$ in. in diameter and graduated in centimetres. 2 cm. of lanoline paste were extruded on to each wound for each treatment. This amount weighed 440 mg. and would supply 4.4 mg. of the active principle from a 1% paste. Treatment followed immediately on wounding and each series of wounds was made in as short a time as possible. In this way variations due to external conditions were reduced to a minimum within a series of wounds. On the other hand, the wounds made at one time were likely to reflect the effect of external conditions on regeneration. In order to avoid possible complications due to the translocation of the growth-regulating compounds within the trees, treatments containing compounds of this type were arranged so that only one compound was applied to each tree.

The form of healing which was obtained with this method is illustrated in Fig. 1 which is a reproduction of the plans drawn in September 1948 of the wounds made in May of the same year. The black area represents the callus tissue and the central unshaded part the exposed wood. The diagrams are orientated as though the branch was running from the bottom of the page to the top.

EXPERIMENTAL RESULTS

In the main the data have been derived from a trial on the variety Lane's Prince Albert. A number of treatments were applied to wounds on each of five trees distributed at random through the plot. The wounds were made and treated at monthly intervals from March 1948 to May 1949, giving a total of fifteen wounds

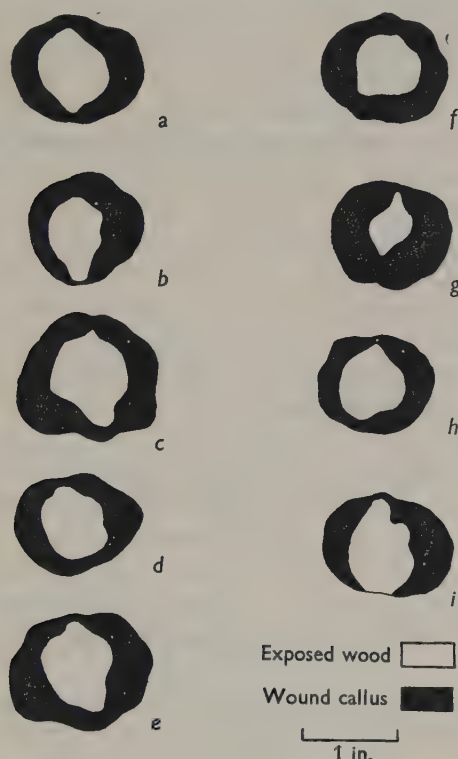


Fig. 1. September: Callus diagrams of wounds made in May: *a*, control, untreated; *b*, control lanoline; *c*, 2:4:6-trichlorophenoxyacetic acid 10%; *d*, 2:4:6-trichlorophenoxyacetic acid 1%; *e*, bis(2-naphthyloxy)acetic acid 1%; *f*, 4-chloro-3:5-dimethylphenoxyacetic acid 1%; *g*, γ -indolyl-3-butyric acid 1%; *h*, cryptonol 1%; *i*, cryptonol 10%.

on each tree. The healing of these wounds was recorded at approximately 2-monthly intervals. The following treatments were included in this trial: 1% γ -indolyl-3-butyric acid, 1% bis(2-naphthyloxy)acetic acid and 1% 4-chloro-3:5-dimethylphenoxyacetic acid (all of which previous experience had suggested would stimulate callus development); 8-hydroxyquinoline sulphate (cryptonol) at 1 and 10% (a fungicide without growth-promoting properties); and 2:4:6-trichlorophenoxyacetic acid at 1 and 10%. The last-named is related chemically to the growth-regulating compounds but more active as a fungicide than those listed above and

also supposedly devoid of growth-regulating activity. The sample of this acid used was found in other tests to have a small growth-regulating activity due possibly to contamination with a more active compound and the results reported with this treatment must be interpreted in this light. The 2:4:6-trichlorophenoxyacetic acid at 10% and the cryptonol treatments were not completely soluble in the lanoline and were applied as suspensions. The trial also included untreated wounds and wounds treated with lanoline alone.

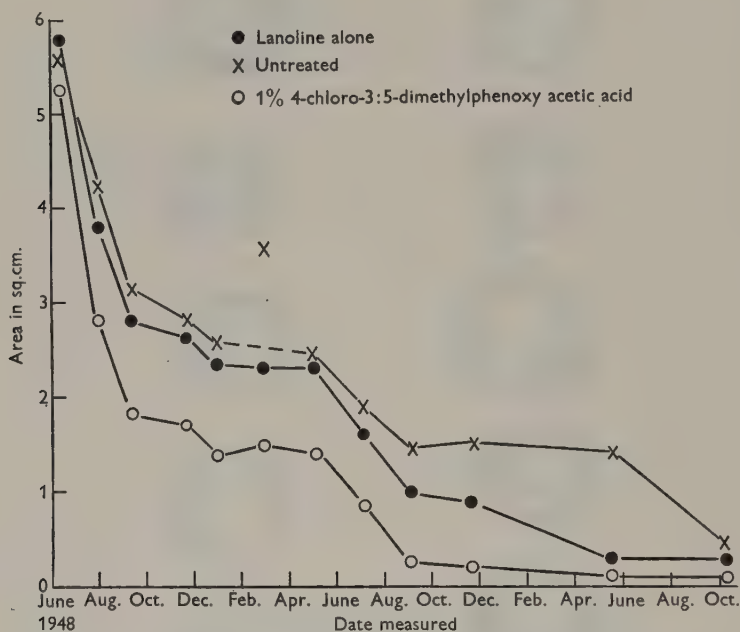


Fig. 2. Regeneration of apple wounds. Effect of time on areas of exposed wood in wounds made during March, April, May and June 1948.

2:4:6-Trichlorophenoxyacetic acid and cryptonol at 10% were both phytotoxic and the damage showed as a narrow dead ring bordering the wound. Callus which had been stimulated with growth-regulating compounds was markedly convoluted when fresh and the final appearance was rougher in treated than in untreated wounds.

Typical wounds made in this trial are illustrated in Fig. 1 and the general course of regeneration is shown in Fig. 2, which illustrates the effect of time on the regeneration of wounds. The wounds, made in March, April, May and June 1948, all behaved in the same way and the data for these months have been grouped. The first area measurement was made in June 1948. Fig. 2 shows the behaviour of untreated wounds and wounds treated with lanoline and 4-chloro-3:5-dimethylphenoxyacetic acid in lanoline. There was a period of rapid regeneration during the summer months between June and September; during the winter, between October

and April, regeneration virtually ceased. The curves suggest that the area of exposed wood decreases slightly over this period but the decrease is not statistically significant. Regeneration starts again with the onset of growth in the spring and the cycle is repeated.

It can be seen from Fig. 2 that the general form of the regeneration curve during the growing season is logarithmic. This curve can be presented in a linear form by plotting the area of wood exposed against the logarithm of the growing time. The linear regression coefficients calculated from these data allow a statistical comparison of the rates of regeneration.

None of the chemical treatments had a significant effect on the slope of the regression line, which suggested that treatment had no sustained effect on the rate of regeneration during the period of measurement. This observation is apparently anomalous since Fig. 2 and the data presented later in Table 3 show clearly that there are significant differences due to treatment in the final sizes of the wounds. In the circumstances this effect can only be the result of differences in the behaviour of the wounds before the observations on healing were started; they might have resulted either from a strong initial stimulation of callus or an earlier start to callus development in the treatments which showed improvement.

With the data available it is not possible to distinguish between these two effects but their operation can be illustrated by calculating the equations which fit the area/log time curves best and estimating from these equations the theoretical date on which callusing started. This series of calculations has been carried out for the healing of the wounds made in March, April and May 1948 during the summer of 1948. There were no observations on the initial size of these wounds after shrinkage had taken place and an average value of 5.5 sq.cm. was chosen. The theoretical dates for the start of callusing calculated in this way are shown in Table 1.

TABLE 1. *Theoretical dates on which callusing started during the 1948 season in wounds made in 1948*

Date wounded ...	5 Mar.	5 Apr.	5 May
Treatment	Callusing started		
Control, untreated	17 June	23 June	17 June
Control, lanoline	21 June	12 June	10 June
Cryptonol, 1 %	16 June	12 June	17 June
2:4:6-Trichlorophenoxyacetic acid, 1 %	22 June	18 June	19 June
bis(2-Naphthyloxy)acetic acid, 1 %	13 June	9 June	17 June
4-Chloro-3:5-dimethylphenoxyacetic acid, 1 %	1 June	24 May	31 May
γ -Indolyl-3-butyric acid, 1 %	25 May	25 May	6 June

This table shows that prior to June the date of wounding has had little effect on the commencement of regeneration. It appears also that the treatment effects recorded could be attributed either to relatively small differences in the times at

which callusing started or to an initial stimulation, equivalent to about 3 weeks' growth at the normal pace.

Joint regression coefficients have been calculated for representative wounds for the 1948 and 1949 growing seasons: these are presented in Table 2.

TABLE 2. *Healing of representative wounds during the seasons of 1948 and 1949*

(Linear regressions of area of exposed wood on log. growing time.)

Date of wounding	Growing season	Regression coefficient
April 1948	1948	-6.12
April 1948	1949	-1.59
December 1948	1949	-4.01
April 1949	1949	-3.78

The regression coefficients are negative since the area of exposed wood decreases as the callus develops. There is no significant difference between the healing rates of wounds made in December 1948 and in April 1949. The wounds made in April 1948 healed significantly faster during the season following wounding than those made in April 1949. The slow rate of regeneration of the April 1948 wounds during the 1949 season is probably largely due to the fact that these wounds were half healed at the beginning of the growing season. A linear extension in callus in these wounds comparable to that shown by the 1949 wounds would produce a far smaller reduction in wood area. A rough correction for this effect was made by multiplying the recorded regression coefficient by the ratio between the area of the 1949 wounds and the area of the 1948 wounds at the beginning of the 1949 growing season. The wounds made in December 1948 were taken as representative of the 1949 wounds and the ratio was $5.46/2.32$. The regression coefficient corrected in this way was -3.74 . This is very much the same as the coefficient recorded for the other wounds healing in the 1949 season.

Since the area of exposed wood during the period of measurement is a linear function of the time after wounding, area measurements of a series of wounds taken at known intervals after wounding give a fair estimate of the rate at which regeneration is taking place. Further, the parallel regression lines noted above suggest that the rate of regeneration is the same for all treatments. In these circumstances comparison of areas of wounds of various ages at an arbitrary time after wounding will also give a fair indication of the performance of the various chemical wound treatments. In the analysis of the results advantage has been taken of both these properties of the regeneration curves.

Table 3 shows the mean area of all the wounds made during the trial as measured on 23 November 1949, at the end of the second growing season. The analysis of these data showed highly significant differences due both to time of wounding and to chemical treatment. The interaction between these two effects was not significant, a result which confirms the previous observation that the effect of treatment

TABLE 3. *Area of exposed wood in wounds made during 1948 and 1949 measured on 23 November 1949.*

Compound		Area of exposed wood (sq.cm.). Individual means													
		Date of wounding, 1948							Date of wounding, 1949						
		Mar.	Apr.	May	July	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	General mean	
Control, untreated		1.25	2.08	1.18	2.63	3.58	3.50	3.10	4.18	3.25	4.25	4.10	3.68	3.06	
Control, lanoline		1.13	1.00	0.53	0.95	2.95	2.20	2.60	2.80	1.80	3.10	3.15	2.90	2.09	
Cryptonol, 10 %		1.03	0.48	1.53	2.15	2.58	2.50	3.03	2.88	3.18	2.63	2.98	2.80	2.31	
Cryptonol, 1 %		0.18	0.30	1.15	0.98	2.60	2.70	2.18	2.48	2.55	2.88	3.48	3.23	2.06	
2:4:6-Trichlorophenoxyacetic acid, 1 %		0.30	0.43	0.50	1.28	2.45	3.05	2.95	2.13	1.95	2.85	2.08	2.85	1.90	
4-Chloro-3:5-dimethylphenoxyacetic acid, 1 %		0.18	0.15	0.28	1.55	2.78	2.45	2.25	1.60	1.43	1.70	2.70	1.95	1.58	
γ-Indolyl-3-butyric acid, 1 %		0.20	0.38	0.23	1.30	3.08	2.43	3.15	2.58	1.75	1.88	2.60	2.48	1.84	
General means		0.57	0.72	0.77	1.55	2.86	2.69	2.75	2.66	2.27	2.75	3.01	2.84	—	

Least significant differences

Probability	Effect (means)		
	Individual	Compound	
		Time of wounding	
0.05	1.09	0.31	0.41
0.01	1.40	0.40	0.53

was confined to the initial stages of healing. Table 3 shows that all the wounds treated with lanoline showed less exposed wood at the end of the trial than the untreated wounds. The wounds treated with cryptonol did not differ significantly from those treated with lanoline alone, while those treated with 4-chloro-3:5-dimethylphenoxyacetic acid in lanoline were significantly smaller than those treated with lanoline alone. The performance of the wounds treated with γ -indolyl-3-butyric acid and 2:4:6-trichlorophenoxyacetic acid was similar and just failed to show as significantly better than those treated with lanoline alone. A growth-stimulating contaminant in the sample of 2:4:6-trichlorophenoxyacetic acid used may have affected the performance of this compound. The final size of the wounds was little affected by the time of wounding during the dormant season, and there was no significant difference in final size of wounds made between October 1948 and May 1949. An exception must be made of the wounds made in February 1949 which healed exceptionally well: this performance must probably be attributed to a purely chance combination of circumstances favouring healing. There is an indication from these data that treatment with growth-stimulating compounds during the season January to May improved healing while treatment during the early part of the dormant season had no effect. To produce a balanced analysis for Table 3 it was necessary to omit some of the observations on wounds made during the growing season; as a result the effect of time of wounding on regeneration during this part of the year is illustrated better in Table 4 in which the balance of the data has been readjusted to include some of the missing readings. Healing became progressively less as the season advanced, and by September it was substantially the same as in the wounds made in the dormant season.

TABLE 4. *The area of exposed wood recorded on 23 November 1949 in wounds made during the summer of 1948*

Month of wounding	...	May	June	July	Sept.	Oct.
Mean area exposed wood (sq. cm.)		0.74	0.93	1.33	3.11	2.73

Treatments with bis(2-naphthoxy)acetic acid at 1% and 2:4:6-trichlorophenoxyacetic acid at 10% were included only in the first season. Wounds treated with the former did not differ from the controls while the latter caused a significant reduction in healing. The actual rate of regeneration was not retarded by the 10% 2:4:6-trichlorophenoxyacetic acid treatment, and the fact that there was significantly more wood exposed in the wounds in this treatment at the end of the season must be attributed to the initial killing of the surrounding tissue.

During the growing season of 1949 a second, less elaborate, trial was carried out on 30-year-old trees of the variety Worcester Pearmain. The trees were wounded and treated in April and the areas measured in November of the same year. Healing in this trial was very poor and the final average area of wood exposed in the wounds measured in November was 4.4 sq.cm. for all treatments. In most cases different

treatments were on different trees and the tree-to-tree variation in healing obscured the small treatment effects. In one series for treatment, however, three graded doses of 2:4-dichlorophenoxyacetic acid were all compared on the same trees and the results of this comparison are presented in Table 5. The areas of exposed wood recorded for the 0.1 and 0.01 % concentrations do not differ significantly and both are significantly smaller than the area recorded for the 0.001 % concentration.

TABLE 5. *The area of exposed wood measured in November 1949 in wounds made in April 1949 and treated with graded concentrations of 2:4-dichlorophenoxyacetic acid*

2:4-Dichlorophenoxyacetic acid concentration (%)	0.1	0.01	0.001
Mean area exposed wood (sq.cm.)	3.67	3.48	4.42

The effect of 1 % 2:4-dichlorophenoxyacetic acid on the regeneration of wounds on Lane's Prince Albert has already been described (Crowdy, 1949*a*). The reaction was violent; immediately round the wound the tissue was killed but beyond the dead zone there was swelling under the bark indicating marked activity in the peripheral tissues. Signs of damage were also visible as malformations of the leaves and flowers on neighbouring shoots. From this trial it would seem that the comparatively poor capacity for regeneration shown by old trees of the variety Worcester Pearmain required a powerful stimulation if any effect is to be shown. This failure to callus is not specific to the variety Worcester Pearmain since the initial callusing trials (Crowdy, 1948) were carried out on 13-year-old trees of this variety.

During the trial on Lane's Prince Albert nearly 600 wounds were made at various times on forty-five apple trees all of which were heavily infected with canker. At the end of the trial the wounds were examined for infection with *Nectria galligena*. Only seven were found definitely infected, while in addition three showed symptoms of disease without bearing fructifications of the pathogen. It would be impossible to draw definite conclusions from such a low rate of infection, but it was noted that the treated wounds were not less susceptible than the untreated and that a fair proportion of the infections occurred on wounds made during the summer months.

DISCUSSION

The healing of wounds on apple trees follows a definite seasonal cycle. It is very active during the growing season from June to September but virtually no healing takes place during the remaining months which form the dormant season. The data seem to suggest that the main period of callus development follows some weeks after the breaking of dormancy in the spring. During the growing season the rate of regeneration appears to be a function of the season rather than of the time of wounding. Healing in 1948 was more rapid than in 1949, and the rate of healing of all the wounds examined in 1949 was about the same, although some of these wounds were made in December 1948 and others in April 1949. There is also some evidence that the April 1948 wounds callused at the same rate during 1949 as the

wounds made in April 1949. The rates of healing were calculated only in selected cases but the non-significance of the area/log time interaction in the general analysis of variance confirms this observation.

A number of chemical treatments have been shown to stimulate the callusing of wounds, but their mode of action remains obscure. It seems clear both from the statistical treatment of the area/log time curves and from the analysis of the measurements of area of exposed wood at the end of the growing season that none of the treatments acts by inducing a sustained improvement in the rate of callusing throughout the whole of the growing season. The improved healing observed may be due either to a marked initial stimulation of growth, or to an earlier start to the healing process. The data do not suggest which of these is the more likely. Similar behaviour has been recorded with shellac treatment (Marshall, 1931). The data also give some indication of the persistence of some of these compounds in the lanoline pastes. None of the wounds treated in December healed more rapidly than the lanoline controls; of the wounds made in January those treated with 4-dichloro-3:5-dimethylphenoxyacetic acid healed better than the controls, suggesting that application 2 to 3 months before growth started was effective. There is little evidence that the actual healing period can be extended markedly by chemical treatment or that out-of-season healing can be stimulated in this way. During periods of active healing it seems that trees with only weak powers of regeneration require stronger stimulation than those which heal more rapidly.

The trials were originally part of an investigation into the effect of stimulating callus development on the spread of *N. galligena* in the peripheral tissues of the apple stems. Initial trials (Crowdy, 1948) suggested that this line of approach offered some prospect of success, and a trial parallel to that described above was laid down on cankers in the field. Each canker was given a single initial treatment. As originally conceived this trial assumed that the effect of the growth compounds was to provide a continuous stimulation to the wound callus and that a strong wound callus would provide an effective barrier to the spread of the pathogen. Later work has shown that neither of these assumptions was justified since the present trials demonstrate that the effect of treatment is confined to the initial stages of callus growth, and continuous stimulation is not provided, and Crowdy (1949*b*) found that the mycelium of *N. galligena* could grow within the fibres which occur in the peripheral tissues of the apple stems and by following this route could pass any callus barrier. In these circumstances it is not surprising that the treatment of cankers produced no useful results, though it is possible that a trial designed to take account of these factors might prove more successful.

The author would like to acknowledge the assistance of Miss P. Tozer and Miss M. Marlow in recording and analysing the results. Fig. 1 has been reproduced from the Report of the Agricultural and Horticultural Research Station, Long Ashton, Bristol for 1948, with the editor's permission.

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EFFECTS OF REPEATED FIELD INJECTIONS OF D-D MIXTURE AGAINST POTATO-ROOT EEL- WORM

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Autumn injections of D-D mixture have been annually repeated for three years on a silt soil at Moulton (Holland, Lincolnshire) and a black fen soil at Prickwillow (Ely, Cambridgeshire), with different results. At Moulton there was an increased yield of tubers each time D-D was used, with no significant residual effects after the first year, and no marked long-term effect on the eelworm population. At Prickwillow D-D gave an increased yield in the first season only, with no positive residual effects on yield, and an apparent stimulating effect on the eelworm population. At Moulton in 1948 and 1950 (but *not* in 1949) the cost of the D-D treatment was heavily outweighed by the value of the resultant increase in crop. However, on the organic soil at Prickwillow D-D treatment was ineffective in 1949 and 1950 and the eelworm population, initially higher than at Moulton, remained at a level inducing failure of the potato crops.

INTRODUCTION

In the autumn of 1947 a number of small field trials were laid down jointly by the West Norfolk Farmers' Manure and Chemical Co-operative Co. Ltd. and by the Agricultural Development Department of Shell Chemicals Ltd. to show the effects of annually repeated injections of D-D mixture on land infested with potato-root eelworm and growing a potato crop each year. None of these trials was extensive enough to constitute an adequate self-contained experiment, but they were regarded by their originators as useful demonstration trials. At that time the results of the trials sponsored by the Agricultural Research Council had not been published (Peters & Fenwick, 1949), but the methods and rates of application were known to the Shell staff, who had co-operated in those also, and were largely followed in the present trials. First injections were made in September 1947, and potatoes were planted the following spring.

In July 1948 the co-operation of the Nematology department at Rothamsted was invited and it was decided to follow up two of the trials, at Moulton and Prickwillow respectively. The West Norfolk Farmers' Manure Co. maintained liaison with the farmers concerned, marked out plots and assisted with weighing the crop on each occasion; Shell Chemicals Ltd. did the annual injections, supplying the D-D and

the injector; Rothamsted collected the soil samples and carried out laboratory estimations of eelworm cysts and larvae per gram of soil, and also handled statistical analyses.

EXPERIMENTAL DESIGN

At Moulton, near Holbeach, the soil is a fine silt. The trial was laid down as two blocks of four plots each, to test rates of application of D-D as follows: 0, 200 lb., 400 lb. and 800 lb. per acre, all at 6 in. lateral spacing. Plots were 200 ft. long and 18 ft. wide, the two blocks being tandem with a 30 ft. headland between them. Each year Majestic potatoes were planted in April.

At Prickwillow, near Ely, the soil is a black fen with a high proportion of peat. There were two blocks of six plots each: two controls, two at 6 in. and two at 12 in. lateral spacing between the injection furrows, all the injected plots receiving D-D at 400 lb. per acre and 6 in. deep. Plots were 210 ft. long and 18 ft. wide, the two blocks being side by side. Each year King Edward potatoes were planted early in April.

It will be seen from the appropriate analyses of variance that very few degrees of freedom are available for testing significance, Prickwillow being superior to Moulton because of the additional replication there. It was realized from the start that only very large differences in eelworm data could achieve significance under such conditions.

TABLE 1. *Analyses of variance (1947-8)*

Source	Degrees of freedom	
	Moulton	Prickwillow
Blocks (<i>B</i>)	1	1
Treatments (<i>T</i>)	3	2
Interaction (<i>BT</i>)	3	2
Error	—	6
Total	7	11

After the crop yields and soil samples had been taken in September 1948, it was decided to split the plots, which were quite long enough to justify this, and to repeat the D-D injections on one subplot of each pair, leaving the other to show residual effects of the original (1947) treatment. At Moulton, after allowing for two 40 ft. headlands between the subplots, this left the latter at 80 ft. \times 18 ft.; similarly, at Prickwillow a 40 ft. headland left subplots of 85 ft. \times 18 ft. The analyses were thereby complicated to the degree shown in Table 2. In addition, the split in the control plots is, of course, a dummy treatment and this has the effect of taking one degree of freedom from the Interaction (*RT*) and adding it to Residual. The control plots are then omitted in computing sums of squares for re-treatment (*R*) and the (*RT*) interaction.

The second injections were made in October 1948; an additional set of spring soil samples was taken in April 1949; the second yields and usual soil samples fell in September and the third injection in October 1949, and the final yields and soil samples in October 1950.

TABLE 2. *Analyses of variance* (1948-50)

Source	Degrees of freedom	
	Moulton	Prickwillow
Main plots*	7	11
Re-treatment (<i>R</i>)	1	1
Interaction (<i>RT</i>)	3	2
Residual	4	9
Total	15	23

* As in Table 1

Unfortunately, an error occurred in the second Moulton injections whereby the 200 lb. per acre subplots were re-injected at 400 lb. per acre, and vice versa. It was therefore decided to repeat this error in the third injections. In the event, these trials were not sensitive enough to be seriously upset by the mistake.

METHODS

Yields were determined by weighing the crop from the middle row of each plot in 1948, and from the middle two rows of the smaller subplots in 1949 and 1950. Soils were sampled with a trowel at points randomly offset to left and right of the mid-line of each plot. The aggregate sample, of 7-10 lb., was air-dried, sifted and mixed, and 200 g. taken for cyst recovery by flotation in Fenwick's apparatus (1940). In the three sets of autumn samples 100 cysts from each were dissected by Hagedorn needle, treated with 1% calcium hypochlorite for 30 min., diluted with water to a constant volume (usually 30 ml.) and agitated to give a random suspension of eggs and larvae, which were counted in 1 c.c. aliquots. From counts of cysts per 200 g. and eggs plus larvae per 100 cysts, eggs plus larvae per g. of air-dried soil are readily calculable. Counts were analysed in logarithmic transformation.

This hypochlorite method is suited to straightforward estimations of eelworm population levels. It is not satisfactory, however, for assessing the kill from a nematicide since killed eggs and larvae cannot be distinguished from living until the former have had time to decay. Accordingly, the samples of spring 1949, which followed an autumn injection without an intervening potato crop, were treated differently. The larvae hatching from batches of 100 cysts in potato root diffusate were collected in formalin and counted by dilution as described in Peters & Fenwick (1949, p. 368). Necessarily, these counts of hatched larvae are considerably lower than the hypochlorite counts of total eggs plus larvae.

RESULTS

Taking the yields first (see Table 3), there are significant treatment effects at both sites in all three years, in spite of the small-scale designs, except at Moulton in 1948 where the data are incomplete and not susceptible of analysis. Here there is a suggestion of almost a ninefold yield increase due to D-D. In 1949 Moulton shows a highly significant yield increase in those subplots re-injected with D-D, no residual effects from the original injections, and no significant differences between the three rates of application. The yield response appears to be less than in 1948.

TABLE 3. *Yields: treatment means**

1948		1949		1950	
Treatment	Tons per acre	Treatment	Tons per acre	Treatment	Tons per acre
Moulton					
0	(0.9)	0-0	1.14	0-0-0	1.78
2	(—)	2-0	0.70	2-0-0	2.15
		4-2	5.68	4-2-2	5.50
4	(7.8)	4-0	1.10	4-0-0	1.45
		2-4	5.58	2-4-4	9.70
8	(7.9)	8-0	1.48	8-0-0	3.05
		8-8	5.40	8-8-8	9.20
Prickwillow					
0	1.79	0-0	1.45	0-0-0	0.71
4	6.06	4-0	0.75	4-0-0	0.55
		4-4	1.45	4-4-4	1.18

* Treatment symbols indicate successive injections in units of 100 lb. D-D per acre, e.g. 2-4-4 represents 200 lb. per acre in 1948, 400 lb. in 1949 and 400 lb. in 1950.

In 1950 the picture is similar: significantly increased yields from the re-injected subplots, no significant residual effects, and no significant difference (in the re-injected subplots) between 400 and 800 lb. per acre; the response from treatment 4-2-2 is, however, significantly less than from the higher rates, though more than from the remaining subplots. The Moulton yields would thus suggest that it is pointless to use a rate as high as 800 lb. per acre, though in some years 400 lb. is superior to 200 lb.; with potatoes grown each year, there is no residual benefit from D-D in the second or third years after injection.

At Prickwillow there was at no time any significant difference between the 6 and 12 in. spacings between injection furrows: those details are therefore omitted from the table. In 1948 D-D gave a highly significant response, with a yield nearly $3\frac{1}{2}$ times that of the controls. In 1949 there was no difference between the controls and the twice-injected subplots, but the once-injected subplots gave a significantly lower yield. This odd result will be commented on when the eelworm counts are considered. In 1950 there was a significant increase in yield in the re-injected plots,

and a bare suggestion that the once-injected plots were still at a disadvantage. It is noteworthy at Prickwillow that the effects of D-D were of economic value only in the first year, after which the best that could be achieved was a mere 1.5 tons per acre.

TABLE 4. *Eggs and larvae per gram of soil. Arithmetic treatment means**

1948		1949			1950	
Treatment	Autumn (hypo-chlorite)	Treatment	Spring (diffusate)	Autumn (hypo-chlorite)	Treatment	Autumn (hypo-chlorite)
Moulton						
0	102	0-0	(38)	112	0-0-0	67
2	175	2-0	(34)	99	2-0-0	51
		4-2	(28)	194	4-2-2	98
4	110	4-0	(26)	114	4-0-0	61
		2-4	(27)	286	2-4-4	79
8	148	8-0	(46)	113	8-0-0	83
		8-8	(48)	169	8-8-8	68
Mean	134	—	(36)	150	—	72
Prickwillow						
0	311	0-0	(144)	328	0-0-0	246
4	354	4-0	(208)	488	4-0-0	225
		4-4	(97)	402	4-4-4	552
Mean	340	—	(150)	406	—	341

* Treatment symbols indicate successive injections in units of 100 lb. D-D per acre.

Counts of eelworm eggs and larvae (or of hatched larvae) per gramme of air-dried soil were analysed in logarithmic transformation. However, the data of Table 4 are given as un-transformed arithmetic means. The site means, or the control values, show how estimations of the population fluctuate from year to year: the big drop in 1950 is especially noticeable. It seems likely that eelworm populations, at or near the failure level as these were, do show considerable variation from year to year, perhaps owing to climatic factors: the control values show these two sites keeping roughly in step each year. It is also clear that the failure level is more than twice as high on the fenland of Prickwillow as on the silt of Moulton, at least on a soil-weight basis.

At Moulton, the autumn 1949 counts show a highly significant increase in eggs from re-injected plots. At both sites there is a suggestion of higher counts from injected plots in 1948, and at Prickwillow the increase in the re-injected plots in 1950 is significant; indeed, the three treatment means for that year are fairly closely correlated with the corresponding yields of Table 3. Thus, a better crop of tubers is accompanied by a larger crop of eelworms.

There is no evidence that the repeated use of D-D leads to a progressive reduction in the eelworm population. Indeed, the significant results (Prickwillow, 1950; Moulton, 1949) show, on the contrary, an increase in eelworm population after using D-D. It is nevertheless noteworthy that the 1949 increase at Moulton is not clearly reflected in 1950. The evidence is that the increase occurs in some years only, and is not necessarily cumulative.

The anomalous yield response at Prickwillow in 1949 can now be considered: the yield from re-injected plots happened to average the same as that from the controls, while the yield from the once-injected plots was significantly lower. Although the eelworm counts in spring 1949 show no significant differences, they suggest a persistence on the once-injected plots of the population augmented by D-D in 1948, whereas the plots re-injected in 1948 have had their population severely reduced. Thus, the potatoes growing in 1949 on the once-injected plots were subjected to a larger eelworm attack which reduced the yield significantly. If this is valid the reduced yield in 1949 on the once-injected plots would be a true residual effect of the original D-D injections. Negative residual D-D effects of this kind have been recorded for *Heterodera schachtii* by Thorne & Jensen (1947).

DISCUSSION

These two trials invite comparison with the extensive A.R.C. trials of 1946-7 (Peters & Fenwick, 1949). In general, they are confirmatory. The increases in both yield and eelworm population, following D-D, have been marked. The lack of any difference between 12 and 6 in. spacing of injection furrows is also confirmed. So is the poor effect on yield in an organic soil, apart from the first-year response at Prickwillow which was exceptional. What is new in the present trials is the repetition of treatments over three years and the possibility of residual effects after the first year. Leaving aside Prickwillow on grounds of soil type, it can be said that at Moulton D-D continued to give an increased crop, at least for three years, and the resulting build-up of eelworm population was neither invariable nor cumulative.

With an eelworm population apparently lower in 1950 than in 1948, the Moulton results justify the brief consideration of economic factors given in Table 5. The 1948 results were incomplete. The value of the increased crop is based on average September prices in each year. The cost of treatment includes both the 400 lb. of D-D and the labour of application, but for present purposes assumes a straightforward injection with no small experimental plots to be dealt with. These values are roughly correct at this one site in these three years. No sort of extrapolation is justifiable at present. Because it has been little used outside of glasshouses hitherto the cost of D-D is abnormally high.

The only significant residual effect in terms of yield is the negative one at Prickwillow in 1949. Grainger (1951) has found positive residual effects in both the second and third years after injection. This may be correlated with the Ayrshire

practice of lifting early potatoes in June, leaving insufficient time, perhaps, for large eelworm populations to build up on the roots of the crop.

TABLE 5. *Financial aspect of Moulton trial (400 lb. D-D per acre)*

Year	1948	1949	1950
				Yields (tons per acre)		
Treated				(7.83)	5.58	9.70
Untreated				(0.88)	1.14	1.78
Increase				(6.95)	4.44	7.92
Value of increase				£52.1	£34.5	£61.4
Cost of treatments				£36.0	£36.0	£36.0
Net gain, per acre				£16.1	-£ 1.5*	£25.4

* Loss.

The authors were greatly indebted to Mr A. Sheppard of the West Norfolk Farmers' Manure Co. for his active assistance and interest, before his sudden death in December 1949. They are also grateful for the large part played by Shell Chemicals Ltd. and their many representatives. The two farmers concerned have endured with much patience the nuisance of a long-term experiment.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Meetings of the Association were held on 3 October, 12 November and 5 December 1952. The full list of speakers, with titles of papers, will be given in the Report of the Council of the Association.

The following are presented below, in slightly abridged form:

Papers read on 3 October 1952

New problems in the control of timber insects. By Dr R. C. FISHER.

The influence of decay in timber on susceptibility to attack by the common furniture beetle, *Anobium punctatum* De G. By Mr J. D. BLETCHLY.

Papers read on 5 December 1952

Wheat yield and soil-borne diseases. By Dr MARY GLYNNE.

A field experiment on wheat infected with eyespot. By Mr G. A. SALT.

Hatching responses in some *Heterodera* species. By Mr R. D. WINSLOW.

NEW PROBLEMS IN THE CONTROL OF TIMBER INSECTS

By R. C. FISHER, *Forest Products Research Laboratory, Princes Risborough, Bucks*

In his presidential address on 'The science and practice of entomology', given to the Zoology Section of the British Association at its meeting in Birmingham in 1950, Prof. Wigglesworth, in discussing control of insects, emphasized 'the increasing scope and need for entomological research to guide intelligently, to supplement and sometimes even to supplant the application of chemical methods'. In no branch of applied entomology is this more true than in forest entomology, especially in relation to insects which attack timber before or after manufacture and use. At a time when trade practices, specifications and numerous other factors are leading to an undoubted increase in damage by wood-boring insects, there is a constant and ever-growing demand for more and better insecticide or preservative treatments for use against species whose habits and especially whose food requirements and relationships are only incompletely known. It is unfortunately true that the expansion of such knowledge by research has been severely handicapped, by research staff having constantly to deal with an ever-increasing amount of advisory work on behalf of the timber-using industries and general public, whilst the need for biological information, the essential foundation of all control measures, remains as pressing as ever: it is pleasing to note that this is becoming more fully realized by the manufacturers of insecticides and preservatives.

It would be unjust to the entomologists concerned to give the impression that their contributions to the solution of many present-day timber insect problems have been negligible. On the contrary, in spite of continued attention to advisory work, which under present circumstances has to be given priority, important advances have been made, and work is in hand which fulfil the requirements of Prof. Wigglesworth's wise and timely comments.

One or two examples of the type of biological data now being sought in relation to the prevention, or at least alleviation, of the losses caused by wood-boring insects will illustrate my point. The first, I take from insects affecting unseasoned wood and often regarded as true forest pests, viz. the ambrosia (pinhole borer) beetles of the families Platypodidae and Scolytidae. Entomologists will recall that these insects are associated with fungi to which the name 'ambrosia' has been given, introduced by the beetles when they bore into standing trees or recently felled logs. In common with other fungi, ambrosia species cannot survive after the moisture content of the timber falls below certain limits. The insects which largely depend upon these fungi for their nutrition are then deprived of their food and gradually die out: seasoned wood is therefore never infested by ambrosia beetles.

These insects are particularly abundant in the tropics, and are responsible for extensive degrade of many tropical hardwoods now reaching the British market. There is an urgent demand by timber suppliers and users for a reduction in losses caused by this type of insect attack, and this has led in many countries to repeated attempts to prevent infestation in logs by superficial treatments with insecticides. Best results have been obtained with some of the newer materials, of which benzene hexachloride is the most promising, but the problem is as yet far from being completely solved. For example, the conditions which invite infestation by ambrosia beetles, either in standing trees or in logs, are not fully understood. Evidence has been obtained from work in Malaya and in West Africa, that there is a close relationship between moisture content of wood and suitability for attack, whilst the presence, or absence and the type of bark on individual tree species also have an important effect. There is, therefore, an urgent need for a detailed investigation of the distribution, habits and even identification of the insects primarily concerned in this problem. An interesting new approach to its solution has recently been suggested by F. G. Browne, Chief Conservator of Forests, Sarawak, who over a period of years has studied these insects in Malaya. Pointing out the existence of some type of attractant to the insects, possibly chemical in origin, associated with fermentation processes which may develop in the sap of unhealthy trees or in felled timber, he emphasizes its importance in determining the comparative susceptibility of different timbers to initial infestation. Preliminary investigations have offered a number of clues on which future work on these lines could be based. A rich and interesting field for further research is opened up which Browne suggests might well result in a practical and economical solution of the ambrosia beetle problem, either by treatments to inhibit attractant production or by the use of small and efficient traps, baited with a strong attractant, in which infestation might well develop in preference to logs in their vicinity.

At present, economic considerations demand the continued use of such insecticide treatments as will give some protection against this type of defect, but there is now every hope of entomological work shortly being started in West Africa to elucidate certain biological aspects of infestation by ambrosia beetles which may point the way to a permanent solution of the problem. At the same time, it is anticipated that more fundamental investigations on the relationships between these insects, ambrosia and other fungi in timber will shortly be started in this country with the object of throwing further light on the nutritional and physiological requirements of these important timber pests.

My second example concerns an insect which infests the sapwood only of comparatively new softwood (coniferous) structural timbers in buildings, viz. the house longhorn beetle, *Hylotrupes bajulus*. Longhorn beetles in general are not pests of structural timbers but are commonly found in unseasoned wood in the forest, and are often associated with decay. The house longhorn beetle is an exception in that it can develop in sound timber partly seasoned, and continue its depredations in dry wood. The present interest of the insect in this country arises out of recent instances of severe damage in localized areas in Surrey and a few other districts in the south of England. This development as an important—if localized—pest of an insect which has long occurred in this country, obviously requires investigation. A study of the records of the occurrence of *Hylotrupes* has revealed two

distinct types of infestation: (1) in old buildings in which activity is no longer present, and (2) in dwelling-houses erected during the past 20-30 years in which infestation is active and damage extensive. In Europe, there is evidence that the house longhorn beetle has become increasingly abundant, and in Denmark and Scandinavia it is regarded as the most important household timber insect. From France and the Netherlands also, there are reports that damage by this insect has recently become more frequent and that it is apparently spreading. It has been introduced to South Africa and is causing such serious infestation in the maritime provinces of the Cape that in an effort to restrict its spread, legislation has been introduced to compel preservative treatment of timber in new buildings in certain areas. At present, we do not have sufficient information on the rate of development, i.e. duration of life cycle, of the insect in this country to form any definite opinion on the risk of spread of infestation. Accordingly, a study has recently been started of the factors determining the suitability of timber to infestation and of the conditions of temperature and humidity favouring its development in the United Kingdom. Is it not possible that here we have an example of an insect which is gradually changing its habit and finding conditions particularly suitable for its development and spread in the type of timber now being used in building projects in this and other countries? These matters require close examination.

Finally, a third example of an insect which also attacks structural timbers, hardwoods as well as softwoods, the common furniture beetle, *Anobium punctatum*, the best known and most widely distributed and troublesome of all timber insects in this country but the most difficult to rear or breed in the laboratory. The explanation is incomplete knowledge of the nutritional and other factors in wood of different species essential for the development of the larvae. My colleague, Mr Bletchly, is dealing with the beneficial effect of some degree of fungal decay in wood for their growth, but, as he will shew, the insect can live and cause considerable damage in timber free from any outward sign of fungal attack, although no doubt traces of fungal mycelium may be present. Atmospheric humidity and its effect on the moisture content of wood appear also to be important factors, but an explanation has still to be found of the widespread belief in this and most other countries that timber is not usually liable to infestation by the common furniture beetle until it has 'aged' or been in use for a period of years. This belief is based on observation, and a number of guesses or estimates have been made as to how old timber must be before damage by this insect may be expected, but data from experiment are not yet available on the changes which appear to be necessary in new wood before it becomes suitable for infestation.

In New Zealand, on the other hand, it has been found that new timber of certain species, e.g. *Podocarpus dacrydioides*, may become infested within a few weeks of felling. The reasons for such widely differing records and experience obviously require investigation, not only biologically but also to determine the type of treatment, and the time at which it should best be applied to prevent this type of insect damage in timber in buildings or furniture.

It has been the practice in some countries to use the larvae of the common furniture beetle for comparing the value of different types of preservative treatments, but at Princes Risborough we have considered it unsound to use this insect in such work until knowledge of the optimum conditions for its development is much more advanced, enabling reliable tests, capable of repetition, then to be set up.

In these very general remarks on some of the biological problems involved in the study of timber insects and their control in this country, I have made only indirect reference to difficulties which arise out of the very nature of timber itself, in relation to entomological work. Timbers of many species are concerned, differing in structure, physical and chemical characteristics and properties, all of which have a close bearing on their susceptibility to infestation. Furthermore, where insecticide or preservative treatments are involved, the permeability of different woods is an important controlling factor which affects their efficacy. Indeed, one of the main problems in timber insect control lies within the field of the biological engineer rather than of the entomologist, and concerns improved methods of application to enable insecticides in the form of liquids or gases to reach the insects and

sometimes even the timber. These problems exist not only in the roofs of buildings but in timber-yards or other premises where stacks of timber are held in store and may be exposed to infestation by insects.

In conclusion, a word on forest entomology in general. It will be apparent from some of the examples I have mentioned that there is no hard and fast line of demarcation between the work of the entomologist interested in the protection of trees and the entomologist concerned with damage by insects in timber. It is true that some insects affect the forest as a growing crop more than its chief product, timber, but as in the case of ambrosia beetles, the health and vigour of a tree, plantation or forest may determine its degree of susceptibility to infestation by a great variety of insects, some of which affect the value of the timber they produce. There is, therefore, every need for the closest collaboration of the forest and timber entomologist in approaching problems which are of mutual interest. Furthermore, the successful solution of many forest and timber insect problems may well demand the co-operation of the botanist, mycologist, plant physiologist, wood chemist and physicist as well as the chemical engineer and wood preserving expert. In all this work, however, accurate knowledge of the identity and biology of the insects concerned remains the basis of successful control.

THE INFLUENCE OF DECAY IN TIMBER ON SUSCEPTIBILITY TO ATTACK BY THE COMMON FURNITURE BEETLE, *ANOBIUM PUNCTATUM* DE G.

By J. D. BLETCHLY, *Forest Products Research Laboratory, Princes Risborough, Bucks*

Advisory records at this laboratory, and the observations of architects and builders, indicate that attacks by the common furniture beetle (*Anobium punctatum*) have increased since the war. One cause might be a consequence of fungal decay following delay in restoration or demolition of war-damaged buildings. However, other factors such as a higher moisture content of timber following fuel shortages, and the storage of furniture during the war in infested premises, have probably also played their part.

An investigation was started at the Forest Products Research Laboratory in 1948 on the effect of decay in wood in relation to the rate of development of the larvae of *A. punctatum* and the length of the life cycle. It was hoped to make a comparison with Fisher's (1941) studies on the death-watch beetle (*Xestobium rufovillosum*), to examine by experiment the observation that damage is often most severe in decayed timber and joinery, and to endeavour to shorten the life cycle to facilitate mass rearing for research and for the evaluation of insecticides and wood preservatives.

Fisher (1941) found that the life cycle of the death-watch beetle is completed more quickly in decayed than in sound wood to a degree related to the extent of decay expressed as a percentage loss in weight of the wood. Decay increased the proportion of nitrogen, and the larvae were able to use most if not all of this additional nitrogen. This factor was thought to be responsible for shortening the larval stage, the nitrogen being rendered more available by the softening effect on the substrate and consequent easier penetration.

No such comprehensive investigation has been made on the relationship between decay in wood and the development of *Anobium punctatum*. Becker (1942), using his larval transfer method, found that when *Poria vaporaria* or *Coniophora cerebella*, causing white and brown rots respectively, produced a loss in weight of 12–15% in his samples of pine sapwood (*Pinus sylvestris*?) the nutritive value was greater than sound timber—but was less with a loss in weight of 20–30%. He considered that in the former case enrichment with a substance, possibly a protein, or increased penetrability of the wood might be responsible, but, in the latter, almost complete destruction of the more easily digested carbohydrates might render the wood less suitable for larval development.

METHODS

The development of *Anobium punctatum* in decayed as compared with sound sapwood was studied in the following materials: (1) freshly converted wood decayed and stored for a period of 7 years, (2) wood decayed after being in store from 14–20 years, and (3) wood decayed immediately after winter felling and exposed for egg-laying in either the following or the succeeding summer. Types (1) and (2) have been termed old material and type (3) fresh material. Differences, if any, between old and fresh wood are not known. The heartwood of types (2) and (3) has also been exposed but not yet examined, and this progress report is confined to discussions on the effect of decay in old and, to a lesser extent, new sapwood. Blocks $2\frac{1}{2} \times 1 \times \frac{1}{2}$ in. were cut from the sapwood of sound timber of known age from the following species: beech (*Fagus sylvatica*), Turkey oak (*Quercus cerris*), English oak (*Q. robur*), walnut (*Juglans regia*), and Scots pine (*Pinus sylvestris*). The initial dry weight was determined, the blocks autoclaved and inoculated with *Polystictus versicolor*, *Phellinus cryptarum* or *Coniophora cerebella* and incubated. Blocks were oven-dried and weighed at intervals so that batches of blocks of fairly uniform and varying extent of decay could be obtained.

To facilitate egg counts and present a similar surface for egg-laying on both sound and decayed blocks, a muslin surface was used as described by Kelsey (1947) and later modified (Bletchly, 1951). Most of the blocks were placed in containers at 22° C. and 75 or 86 % relative humidity, but some were kept out-of-doors. The blocks were exposed to egg-laying by beetles (approximately two pairs per block) and arranged so that the insects had choices of various combinations of sound and decayed material and of different species of wood. Later, the number of eggs laid was counted and the total apparently successful larval penetrations determined by external inspection. Finally, after some 20 months (15 months for walnut) from egg-laying, although no emergence had taken place, the blocks were cut up and the live larvae removed and weighed.

RESULTS

Suitability of decayed wood surfaces for egg-laying. It was found that females pushed their eggs into all surfaces of decayed wood blocks to a depth depending on the extent of decay, so that, in the more heavily decayed material, unless a muslin technique had been used, it was not possible, without damaging the sample, to count the eggs. In the sound controls very few eggs were laid except on the rougher end-grain surface unless muslin was provided (Kelsey, 1947).

Larval penetration. A small amount of decay increased successful larval penetrations across the grain compared with the controls when the muslin technique of Kelsey was used. With the later modified technique (Bletchly, 1951) ensuring that egg-laying occurred only on the end-grain, most of the larvae penetrated successfully into sound wood.

Rate of larval growth in decayed wood. Although both white and brown rots produce a loss in weight in wood proportional to their growth, their physiological effect differs (Cartwright & Findlay, 1946), and this could affect the suitability of wood for attack by wood-boring insects.

It was found that there was a much greater gain in weight by larvae in beech, Turkey oak, Scots pine and walnut decayed by *Polystictus versicolor*, than in the sound controls. The gain in weight is related to the extent of decay, but there are indications that it reaches a maximum in moderately decayed wood and may be less in more heavily decayed material. In Scots pine, a loss in weight of only 8 % was sufficient to cause an increase in the rate of larval growth.

Although there is less information on the effect of *Coniophora cerebella*, it was found in beech that if the average larval weight is plotted against the percentage loss in weight by decay, the curves for white and brown rots are very similar under incubator conditions for

the lower losses in weight. Comparable data are not available for the higher losses in weight caused by brown rot.

Survival of larvae in decayed wood. Data have also been obtained on the proportion of larvae surviving in decayed wood compared with that in sound wood, assessed by the number of tunnels apparently penetrating into the block. This criterion was adopted rather than the number of eggs laid, in order to exclude additional variables such as the percentages of unhatched and infertile eggs.

In general, it seems that the survival rate of larvae in wood decayed by white rots is lower than that in sound wood, but in Scots pine, in an incubator, decayed to approximately 8% loss in weight, and in walnut out-of-doors decayed to approximately 29% loss, the survival rate was higher than in the controls. It also appears that the survival rate is much lower in wood decayed by brown than by white rot.

DISCUSSION

In the older material, the interim results from four hardwoods and one softwood are similar and may be examined collectively. Decayed wood is more suitable than sound wood for egg-laying and for initial larval penetration and in wood attacked by the white rots investigated, the larvae grow more quickly and thus inflict greater damage, although fewer may survive. This rapid growth may be due to the presence of additional nitrogen and greater ease in boring, but both Becker (1942) and Spiller (1951) have shown that cellulose forms an important constituent of the diet. Wood attacked by brown rot appears less favourable for larval development than that decayed by white rot. Loss in weight by brown rot mainly represents a fall in the cellulose and associated pentosan content, whereas loss in weight by white rot represents a fall in all the constituents of wood, including the lignin. Thus, relatively more cellulose is available in wood decayed by white rot than in wood decayed by brown rot to a corresponding loss in weight. It is possible that larval growth is governed by a lower critical limit for a constituent of wood such as cellulose below which the content must not be reduced and that this limit is reached relatively quickly by the action of a brown rot. In addition, wood decayed by *Coniophora* is more friable with a greater tendency to shrink and crack than wood decayed by a white rot and thus appears to be mechanically less suitable for boring. The higher rate of growth of larvae and greater damage inflicted in decayed wood is somewhat offset by the lower survival rate and it is possible that, amongst other factors, overcrowding plays some part.

Apart from the similarities already noted, these results show differences from the relationship between decay and attack by the death-watch beetle. The common furniture beetle infests and breeds successfully in sound timber which Fisher (1941) has shown to be less suitable for the death-watch beetle. Further, although decay increases the rate of larval growth of the common furniture beetle, it does not appear to shorten the life cycle as with the death-watch beetle; in fact, in an incubator, larvae have continued to grow to a large size without pupating, e.g. a weight of 17.5 mg. has been recorded after 20 months in beech decayed by *Polystictus versicolor*.

Although none of the decayed heartwood blocks exposed for egg-laying has yet been examined, observations in buildings have shown the effects of fungi (not always wood-destroying types) in facilitating attack by *Anobium punctatum* in the heartwood: as this insect is normally confined to sapwood (at least in those species where the sapwood and heartwood are distinct) this is of structural importance. Decayed heartwood may indeed be preferred to sound sapwood; for instance, in a board of rowan (*Sorbus aucuparia*) attack in the heartwood decayed by *Armillaria mellea* was severe but slight in the sound sapwood.

The effect of decay on the susceptibility of freshly seasoned sapwood has been less fully investigated. In the incubator, no larvae survived in fresh Scots pine whether sound or decayed by *Coniophora cerebella*; however, larvae survived in English oak decayed by the

white rot, *Phellinus cryptarum*, but not in the sound controls. Out-of-doors, beetles emerged in the summer of 1952 from freshly seasoned English oak decayed by the latter fungus to 36 % loss in weight and from the sound controls—a life cycle of 3 years in each case. Although no emergence has yet taken place from the incubator material, the results obtained there indicate a shorter life cycle in wood decayed by *P. cryptarum*.

Some exploratory experiments on walnut inoculated with the moulds *Paecilomyces varioti* and *Chaetomium globosum* have indicated that although larvae do not appear to develop more rapidly than in the controls yet the percentage surviving is greater.

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WHEAT YIELD AND SOIL-BORNE DISEASES

BY MARY D. GLYNNE, *Rothamsted Experimental Station, Harpenden, Herts*

The study of soil-borne diseases of wheat, in which we have been engaged for the last 15 years, helps to throw light on some of the reasons why different crops of wheat yield so differently and respond so differently to fertilizers.

Disease surveys of winter wheat, made in many parts of Britain and at Rothamsted, provided problems on which we experimented with plants grown in small pots in the glasshouse, in large pots outdoors and in the field (similar experiments in pots and field often running at the same time). The surveys showed that the two soil-borne diseases, take-all (*Ophiobolus graminis*) and eyespot (*Cercospora herpotrichoides*) are outstandingly important; both depend so much on previous cropping that the likelihood of severe attack in a wheat crop can be assessed from a knowledge of the sequence of cropping in preceding years, the type of soil and general climatic conditions. For instance, about one wheat crop in three suffers severely from eyespot on the heavier land when grown in the 4-course rotation in East Anglia, one in two suffers in the wetter north of England.

Loss from take-all is very obvious and is most severe when plants are inadequately fed, because well-nourished plants produce new roots to replace those damaged by the fungus.

When the number of plants per unit area decreases, more nutrients are available to each plant and so take-all has less effect. This is clearly shown in pot and field experiments with varying seed rate and varying amounts of sulphate of ammonia (basic phosphorus and potassium being supplied). The effect of take-all was almost eliminated and grain yield increased by reduction in seed rate in the same way as it was by addition of the fertilizer. Thus take-all, because it reduces the yield of poorly nourished plants very much and of well nourished ones very little, increases the response in grain yield to fertilizers.

In the field take-all is often controlled by 1 and generally by 2 years free from the susceptible crops wheat and barley; and even when wheat is grown continuously, effects of the disease are decreased by sound nutrition. The serious losses now suffered in many parts of Britain from take-all are therefore unnecessary. Eyespot is much more difficult to control because generous manuring does not overcome it and, though one year free from susceptible crops generally reduces its incidence, it does not do so enough to prevent serious loss; for this 2 years are needed.

The extent to which eyespot reduces yield varies much under different conditions; in one pot experiment, loss from eyespot varied from 19% in well nourished to 86% in starved plants; in another, loss increased from 22 to 45% with increasing plant number. In a series of experiments made over several years, loss from eyespot under various conditions averaged about 33%; or expressed differently, controlling eyespot added 50% to the yield. General lodging, which increased with increasing straw weight and with the percentage straws severely infected, is another source of loss in the field, but rarely occurs in pot experiments.

In these and other experiments in which control and inoculated plants are compared, the effects of eyespot and take-all are such as to emphasize their probable importance as factors influencing yields in the field. The incidence of both diseases on wheat grown in field experiments at Rothamsted has been determined in annual surveys since 1938, and will now be considered in relation to the wide variation in yield which occurs under similar weather conditions.

On Broadbalk field, wheat (Squarehead's Master) is grown with various manurial treatments for 4 years after 1 year under fallow. In the 10-year period 1938-47 wheat grown 2, 3 and 4 years after fallow showed the effect of nitrogenous fertilizer by the increase in mean grain yield from 11 cwt./acre with minerals only to 22 cwt./acre when the heaviest dressing of sulphate of ammonia was added. Corresponding figures in the first crop after fallow were 21 and 27 cwt./acre respectively.

One year under fallow increases weight of straw and decreases the incidence of eyespot, the first effect increasing, the second decreasing the tendency to lodge. Sometimes one factor, sometimes the other, predominates, so that in some years there is more lodging in sections after crop, in others after fallow. Eyespot therefore, unlike take-all, reduces the response to fertilizer because, at the higher levels it increases lodging.

The average area lodged (1938-47) was about the same in sections after fallow and after crop in each plot, so that the effect on lodging cannot account for the consistently higher yield obtained in the first crop after fallow. This difference, while partly due to increase in available nitrogen in the less fertile plots and partly to the suppression of weeds is also probably partly due to reduction in straws severely infected by eyespot from 48 to 26% (mean plots 5-8). Take-all was noticeable in some years in plots deficient in nitrogen and its control by fallow may have contributed to the increase in yield in these plots.

In the 4-course rotation on Hoos field, wheat (Yeoman) and barley (both susceptible crops), are each grown once in 4 years, and in the 6-course rotation on Long Hoos, once in 6 years. In the 10-year period, 1938-47, they yielded 18 and 31 cwt. grain/acre respectively. Eyespot was not severe enough in either experiment to cause lodging of the amount of straw produced, and take-all was negligible. The difference of 13 cwt./acre in their grain yields, while partly due to difference in soil fertility, is also likely to depend partly on the difference in incidence of eyespot, 31% of the straws being severely infected at harvest on the 4-course, only 12% on the 6-course.

In a 3-year experiment described by Glynne (1951), spraying the sixth successive crop of winter wheat with H_2SO_4 in autumn and spring increased weight of straw with some treatments by as much as 19 cwt./acre, but reduced the incidence of eyespot so much that it almost entirely prevented lodging, thus enabling the crop to respond to higher doses of sulphate of ammonia. Yield of plots which received no sulphate of ammonia was reduced by a severe attack of take-all (which was not affected by spraying), and this also increased response to the fertilizer. Thus 4 cwt. sulphate of ammonia increased yield by 9 cwt./acre on sprayed plots, but by only 2 cwt./acre on unsprayed where lodging prevented response at the higher levels.*

Both spraying and fallow decreased weeds, an effect which doubtless affected yield; but in other experiments in which weeds were few, increases in yield with decreases in severity of eyespot and take-all were of the same order as those shown above, and suggest that disease incidence played a large part in determining yield.

The value of thin seeding on eyespot-infested land was shown in 1946 and 1948 (Glynne, 1951). Besides reducing the severity of take-all, thin seeding also reduced eyespot by enabling plants to produce more tillers which delayed infection of ear-bearing straws and by promoting evaporation round bases of the wider spaced plants. Seed rate had little effect on straw yield, but thin seeding reduced eyespot and therefore lodging. Each additional bushel of seed increased lodging by about as much as an extra 2 cwt. sulphate of ammonia applied in March.

Land badly infested by eyespot, take-all and weeds was used for a rotation experiment; four parallel strips were sown with four different crops in the first year, and in the second with four crops in strips at right angles to the first. The resulting 16 plots were sown with wheat (Squarehead's Master) for the 1951 harvest. With similar manurial treatments but different crops grown in the two preceding years, yields in 1951 varied from 21 to 39 cwt./acre. Plots in which wheat followed wheat or barley yielded an average of 24 cwt./acre, 32 % straws being severely infected by eyespot, take-all was found on roots of about 16 % plants in April but $2\frac{1}{2}$ cwt. sulphate of ammonia applied later partly controlled the disease so that only 2 % showed severe symptoms at harvest. Wheat following non-susceptible crops produced an average yield of 35 cwt./acre with only 8 % straws severely infected by eyespot, and no signs of take-all.

Yields exceeding 40 cwt./acre have sometimes been obtained at Rothamsted, but only where Squarehead's Master has been replaced by newer varieties. In an experiment on residual effects of organic manures applied to the preceding potato crop, Jubilegem in 1945 gave yields ranging from 29 to 41 with a mean of 34 cwt./acre with 4 % straws severely infected by eyespot. With the same treatments and similar low disease incidence Bersee in 1949 yielded 41-47, mean 44 cwt./acre. But in another part of the same field with the same treatments, Bersee in 1950 yielded only 22-29, mean 27 cwt./acre, because the preceding potato crop had (in this instance only) followed three consecutive wheat crops which had increased disease so that the Bersee in 1950 had 42 % straws infected by eyespot as well as some with take-all.

Field observations seldom provide conclusive evidence. But the consistent association in the field of high yield with low incidence of eyespot and take-all, together with the effects observed in controlled experiments, support the view that these diseases have a major effect on yield comparable with that exerted by fertilizer and by variety.

Thus by growing a high yielding variety in a good crop rotation, with sound nutrition, yields exceeding 40 cwt./acre have been obtained at Rothamsted. This is about twice the average yield of wheat in Britain. Rothamsted has only average wheat land, and although some wheat is grown on lighter poorer soil, most is grown on land as good or better than that at Rothamsted. It thus seems likely that if the knowledge available is used the average yield of wheat in Britain can be increased by at least 50 %.

* Mean of plots sown with $2\frac{1}{2}$ and $3\frac{1}{2}$ bushels/acre.

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A FIELD EXPERIMENT ON WHEAT INFECTED WITH EYESPOT

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This paper summarizes the main effects at harvest of cultural treatments applied to wheat sown in October 1951 on eyespot-infested land at Rothamsted.

Treatments

Variety. (1) Squarehead's Master 13/4; (2) Bersee.

Seed rate. $1\frac{1}{2}$ and 3 bushels per acre.

Nitrogen. (a) Rate of application: sulphate of ammonia applied at 0, 2 and 4 cwt./acre.

(b) Date of application: 24 October 1951, 13 March 1952, 15 April and 22 May.

Spraying. Sulphuric acid ($12\frac{1}{2}$ % BOV by vol.) sprayed at 100 gal./acre on 14 March 1952.

The factorially designed experiment consisted of 80 plots, each $\frac{1}{50}$ acre, arranged in 8 blocks of 10. It repeated some treatments previously tested (Glynne, 1951), but new ones included the application of sulphate of ammonia at four different dates; and the use of the shorter, stronger-strawed variety Bersee as well as Squarehead's Master. Spraying with H_2SO_4 was done in spring only.

The effect of treatments

(a) *Spraying with sulphuric acid.* Spraying had little effect on the weight of straw, but greatly reduced both the incidence of eyespot and lodging; it controlled weeds and increased yields by an average of 10 cwt./acre. In Squarehead's Master spraying reduced the proportion of straws with severe eyespot from 66 to 17 %, reduced the area lodged at harvest from 95 to 36 %, and increased the yield of grain from 22 to 32 cwt./acre. In the variety Bersee it reduced severe eyespot from 56 to 15 %, lodging from 54 to 1 % and increased yield from 33 to 44 cwt./acre. (An increase of 3 cwt./acre would have covered the cost of spraying at present-day prices.)

(b) *Variety.* There was little difference in eyespot incidence in the two varieties, but Squarehead's Master produced more straw (averaging 66 cwt./acre) than Bersee (55 cwt./acre) and so lodged earlier and had a greater area lodged at harvest. Bersee produced an average of 10 cwt./acre more grain than Squarehead's Master; the increase was only partly due to the smaller area lodged.

(c) *Rate of sowing.* The effect of varying seed rate affected both varieties similarly. Reducing it from 3 bushels (the normal rate at Rothamsted) to $1\frac{1}{2}$ bushels/acre had relatively little effect on the weight of straw, but reduced the percentage of severe eyespot by an average of 17 % in unsprayed and 9 % in sprayed plots, thus delaying lodging and decreasing the area lodged at harvest. Thin seeding increased yield by 5 cwt./acre in unsprayed blocks, but increased it by only 2 cwt./acre in sprayed blocks, where there was less eyespot.

(d) *Rate of application of sulphate of ammonia.* Applying sulphate of ammonia had little effect on the percentage straws with severe eyespot, but increased the weight of straw and so accelerated and increased lodging. Its effect on yield increased as the percentage area lodged decreased. When applied to unsprayed plots of Squarehead's Master, which became severely lodged by harvest, it increased yield by only $\frac{1}{2}$ cwt./acre, but when applied to sprayed plots of Bersee, which did not lodge, the single rate of application increased yield by 6 cwt./acre and the double rate by $10\frac{1}{2}$ cwt./acre.

(e) *Date of application of sulphate of ammonia.* The date when sulphate of ammonia was applied had little effect on eyespot incidence but determined the weight of straw and therefore the amount of lodging. Applications in March and April, by accelerating and increasing lodging, gave smaller increments in yield than application in October, except when lodging was controlled by spraying in the variety Bersee. Then applications in October, March and April all gave similar high yields, May application gave rather less.

Thus, the main effects of treatments on the yield of grain were as follows: spraying increased yield by an average of 10 cwt./acre in both varieties; Bersee yielded an average of 10 cwt./acre more than Squarehead's Master; reduction in seed rate increased yield by 5 cwt./acre in unsprayed, and by 2 cwt./acre in sprayed plots. Responses to the application of 4 cwt./acre sulphate of ammonia ranged from $\frac{1}{2}$ cwt./acre in unsprayed plots of Squarehead's Master, which lodged badly, to $10\frac{1}{2}$ cwt./acre in sprayed plots of Bersee which did not lodge.

Yields ranged from $16\frac{1}{2}$ cwt./acre in an unsprayed, thickly sown plot of Squarehead's Master, to 49 cwt./acre in a thinly sown plot of Bersee which was sprayed and had received 4 cwt./acre of ammonium sulphate; this equals the highest yield of wheat ever recorded at Rothamsted.

The experiment has been sown on the same site for a second year, and a third year is intended, to compare the effects of the same treatments on successive wheat crops in different years.

HATCHING RESPONSES IN SOME *HETERODERA* SPECIES

By R. D. WINSLOW, *School of Agriculture, University of Cambridge*

Using techniques evolved by Fenwick and his colleagues of the Nematology Department at Rothamsted, hatching responses of beet, cabbage, *Galeopsis*, clover, pea, oat, carrot and hop-root eelworms were investigated.

The hatching curves of beet, cabbage, *Galeopsis* and hop eelworms were examined and were found similar to that described by Fenwick (1950) for potato-root eelworm.

Beet eelworm hatched freely in leachings from hosts such as sugar beet, mangold, and the commonly grown crucifers but, in general, hatching responses of this species in non-host leachings were not marked, being often less than the responses in the water controls. However, leachings from swine-cress (*Coronopus squamatus*) stimulated hatching equal to that of leachings from good hosts, although Jones (1950) had found that cysts of beet eelworm apparently never formed on this plant despite the fact that the roots were invaded by the larvae. This plant is therefore a potential agent for the biological control of beet eelworm.

Hatching in cabbage-root eelworm was not stimulated by leachings from swine-cress, which is a host of this eelworm. Thus, it appears that this interesting crucifer stimulates hatching in beet eelworm, of which it is not a host, but does not stimulate hatching in cabbage-root eelworm, of which it is a host. Furthermore, no other crucifer is known which is a host of cabbage-root eelworm but not of beet eelworm. For this reason swine-cress has a further possible use as a test plant to detect the presence of cabbage-root eelworm in mixed soil infections.

From various experiments involving cabbage-root eelworm, it was found that this species hatched readily in leachings from all *Brassica* spp. tested (Brussels sprout, swede, rape and black mustard, all of which are hosts) but not in leachings from cruciferous hosts other than *Brassica* spp., namely white mustard, cress, swine-cress, treacle mustard and shepherd's purse.

Galeopsis-root eelworm was found to hatch readily in pea leachings but not in leachings from the hosts red clover and *Veronica* sp. Clover root eelworm behaved similarly, giving

a high response in pea leachings, but not in leachings from red clover. In host range tests at Cambridge, cysts of neither eelworm have been found on pea roots, although Franklin (1951) found that clover-root eelworm could reproduce on pea, but only to a limited extent. The similarity in the behaviour of these two eelworms in hatching experiments supports the belief of Jones (private communication) and Franklin (1951) that they may be synonymous.

An attempt was made to apply the knowledge gained from these experiments, in bio-assay of mixed soil infections. Quantitative analysis, in terms of 'hatchable' larvae, of an artificial mixture of beet-, cabbage- and *Galeopsis*-eelworm soils was tried, using a sequence of root leachings, namely mangold, swede and pea leachings. The response in each phase was much as expected, beet-eelworm larvae apparently emerging in the mangold leachings, cabbage-eelworm larvae in the swede leachings, and *Galeopsis*-eelworm larvae in the pea leachings. Thus, for these three eelworm species, good results, on a 'hatchable' larvae basis, could be expected.

In other experiments, carrot eelworm hatched only in leachings from the one known host, carrot, and hop eelworm hatched only in leachings from representatives of the Cannabinaceae (hop and hemp) and Urticaceae (small nettle and pellitory). From this it is thought that the above method may be extended to include these two species. In contrast, in pea- and oat-root eelworms, negligible responses were obtained in host and non-host leachings. Thus the method in its present form is not applicable to bio-assay of these important pests. It may be that the physical conditions, under which the experiments described above have been carried out, will have to be altered to suit the requirements of these two species.

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REVIEWS

Back-Crossing. By MARY THOMAS. Commonwealth Bureau of Plant Breeding and Genetics, Cambridge. 1952. 15s.

The introduction by crossing into otherwise desirable plant strains or species of such characters as resistance to a disease, or a parasite, high fibre strength, low nicotine content and others, frequently results in the loss of those valuable properties for which the 'recipient' strain had been selected. By repeated back-crossing to this strain and simultaneous selection for the introduced character—directly for dominant traits and by test-crossing for recessives—this loss of quality can be obviated; in theory at least any character dependent on one or not too many genes may thus be incorporated into an otherwise unaltered homogeneous strain originally lacking this character. While successes of back-crossing in cereals are generally known, similar results in other crops have been less publicized. The present monograph dealing with back-crossing methods in cotton, tobacco, tomatoes and potatoes will therefore be welcomed by many plant breeders and by agriculturists and biologists in general.

H. KALMUS

The Principles of Line Illustration. By L. N. STANILAND. Pp. 212 with 161 illustrations in text. London: Burke. 25s.

In our fraternity we know Mr Staniland as an entomologist, an artist, and a deviser of mechanical contrivances in the very tradition of Leonardo himself—witness his square-wheel dusting machine. We have enjoyed his *Let's All go Sketching*, *Let's Understand Art* and *Now we'll Try Oils*, not only for the charm of his illustrations, but because, unlike so many books on how to paint or draw, they are not mere one-man exhibitions of prowess, but real attempts to persuade others to try their hands at these arts.

Now this most useful book, on line illustration especially for biologists and other scientific workers, is devised and written in the same spirit. It is an introduction to many fascinating techniques, with examples of their use on zoological and botanical subjects, from the simplest working sketch of a leaf or the silhouette of a caterpillar, to a detailed and finely modelled drawing of a skull or of the whole inflorescence of a plant. It shows how many difficulties may be overcome by the use of scraper board, splatter, mechanical tone, or a little process white. It has something interesting on every page, but, above all, it gives the help that so many of us have long wished to have on the treatment of outline and shading, texture and shadow, so that our drawings for purposes of record, or for the illustration of our papers, may be, if not works of art, at least accurate and pleasing representations of what we want to show.

All means are fair to this end, and an important part of the book is devoted to mechanical aids to accurate drawing, including dodges with tracing paper, an improvised camera lucida costing nothing, and the ingenious glass-tracing perspective apparatus first described by the author in these *Annals* (33, 170-7, 1946). Mr Staniland has now developed and extended the range of usefulness of his apparatus, notably by the incorporation of lenses, and this book contains full details of the apparatus itself, together with many examples of drawings made with its aid in the field and in the laboratory.

There is a good section on the preparation of graphs, charts and diagrams, and although the book does not deal with mechanical draughtsmanship, its sections on the making of rapid and accurate perspective drawings of models and machinery will not fail to interest the 'visual-aids' man, the commercial artist, the designer and the engineer.

E. C. LARGE

Medical Mycology: an Introduction to its Problems. By G. C. AINSWORTH. Pp. ix + 105, with 7 text-figures and 8 plates. London: Sir Isaac Pitman and Sons. 1952. 15s.

The publication in this country of a work on medical mycology is a rare event: indeed this is the first book to survey the field in Britain since W. Tilbury Fox published his *Skin diseases of parasitic origin* in 1863. In the intervening 90 years the scope of medical mycology has been widened by the discovery of systemic mycoses and of fungus allergy, but the light which shone in Britain now shines more brightly in North and South America. The recent revival of interest in Britain following the surveys by Dr J. T. Duncan should be helped by Dr Ainsworth's admirable little book.

The author sets out to build a bridge between two island disciplines, that of the clinical and that of the systematic mycologist. Each chapter deals with one of the main topics of medical mycology in a straightforward manner, then the argument turns through a right angle as it were, and the topic is shown to illustrate one of the peculiar complex problems with which medical mycology is beset. The design of the bridge is 'willow pattern' rather than 'Bailey', but because of this it affords an excellent view of the landscape.

The reader will not find here a compendium of facts, telling him how to take samples of tissue, identify the fungus, diagnose the disease, and cure the patient. Instead he will imbibe the authentic flavour and philosophy of the subject, its history, development and background, illustrated by the main types of disease resulting from the activities of fungi within the human body.

The contents of the chapters include: I, a general introduction on the significance of fungi in various types of mycosis; II, the ringworm fungi and mycoses of the skin, with a summary of the principles of botanical nomenclature for the instruction of the medical worker; III, an account of the mycoses of the respiratory tract, and of the difficult problem of deciding whether yeasts found in sputum or lungs are in fact pathogenic; IV, an account of two diseases, Madura foot and chromoblastomycosis, either of which may be caused by one of several different fungi; V, an account of the systemic mycoses, briefly summarizing the rapidly increasing knowledge of the systemic mycoses on the American Continents; VI, an explanation of serological methods in diagnosis, with a condensed account of the complement-fixation technique, for the instruction of the pure mycologist; VII, a survey of the role of fungus spores as allergens, including some original data; VIII, a brief account of fungus poisoning.

There is a bibliography of about 150 titles, and the illustrations are excellent and well chosen.

P. H. GREGORY

Poplars. By T. R. PEACE. Pp. 50, with 49 Plates. Forestry Commission Bulletin No. 19. H.M. Stationery Office. 1952. 7s. 6d.

The publication of a revised bulletin on poplars by Mr T. R. Peace with the assistance of specialists comes at an appropriate time. Many land owners are anxious to plant poplars and this publication will enable them to profit by the experience gained in Great Britain during the past thirty years.

There are fifty pages of text and twenty-four pages of plates. Some twenty-five pages of text are given to silviculture, twenty-three to pests and diseases and four to the properties and use of the timber. The chapters on the choice of varieties and on diseases are the king pins for the successful culture of the crop. A key for the identification of the chief poplars in Great Britain is provided which is an achievement in view of the difficulties associated with the systematics of this genus. Detailed information is given on the choice of planting site, propagation of sets, planting and maintenance. The life histories of the principal

insect pests are described but lack of information on the methods of control and particulars of insecticides is disappointing. The economic status of pest-control measures could with advantage be enlarged upon. The chapter on timber shows that well-grown trees have a number of special uses and it is to be hoped that the bulletin will encourage greater planting of this valuable crop.

The plates are well selected and of outstanding quality and their large size and the use of good paper does full justice to the skill of the photographers.

Every forester and all interested in trees should purchase a copy for the modest cost of 7s. 6d.

H. G. H. KEARNS

Statistical Method in Biological Assay. By D. J. FINNEY. Pp. xix + 661. London: Charles Griffin and Co. Ltd. 1952. 68s.

The technique of biological assay is one that has developed rapidly during the past twenty-five years. Recently, a stage of consolidation has set in. The basic principles of assay technique are now widely agreed upon, and much of the essential statistical knowledge has been worked out. The time is fully ripe for a detailed discussion of the whole topic, and Dr Finney, who himself has been responsible for many advances in this field, has filled this need in a quite admirable way. His book is readily understandable without evading difficulties of principle, and it covers all the important aspects of the subject.

The book is aimed at those who are already fairly familiar with less specialized techniques of statistical analysis and with the principles of experimental design. This should not frighten off the biologist without formal mathematical training; Dr Finney gives, in fact, four suggested courses of reading selected from the whole book, one of which has this kind of user in mind, although he suggests a preliminary study of the regular statistical text-books. Every analytical procedure is illustrated by numerical examples worked in considerable detail, and the non-mathematical biologist should not have great difficulty in discerning the purposes behind the various operations or in applying them to his own problems.

The book opens with a general discussion of biological assays and the use of standard preparations, pointing out that only so-called dilution assays employing such standards are to be considered. We then meet the simplest type of assay in which the dose needed to produce a fixed response can be exactly determined. Such assays (the cat assay of digitalis is an example) are not particularly common, but they provide an admirable peg on which to hang a full discussion of the basic statistical techniques and terminology. We meet at once the idea of relative potency, and in determining the precision of an estimate of this quantity Fieller's theorem on the fiducial limits of a ratio is naturally quoted, with two analogous results employing the Behrens-Fisher distribution. Dose metameters and covariance adjustments are also introduced.

The stage is now clear for the introduction of the more common indirect type of assay with a quantitative response. The discussion proceeds from the general to the particular; chapter 3 treats the important preliminary stage of devising a new assay; chapter 4 the general analysis of parallel line assays; chapter 5 the routine analysis of properly designed assays. Chapter 6 discusses the economics of these designs and the principles to be borne in mind when setting them up. Chapters 7 and 8 repeat this programme for slope-ratio assays. Incomplete block designs are treated in chapter 9, many of them employing a type of confounding particularly adapted to assays, while chapter 10 gives a full description of 'cross-over' designs, in which each test-subject is used more than once. Chapter 11 extends some of these ideas to the simultaneous assay of several test preparations.

Chapter 12 deals with the use of concomitant information by means of covariance adjustments, and chapter 13 with the analogous problem of combining two or more alternative responses into a single measure of response by discriminant techniques. Chapter 14 deals

with the important problem of combining the results of independent assays; there is also a discussion of the heterogeneity of the error estimate of assays carried out in blocks. This topic might well have been treated earlier, since appropriate modifications will often be required in the routine analysis of such assays. The next two chapters are also of an advanced nature, dealing with the interpretation of validity tests and with the analysis of assays for which no simple metametric transformation can be found.

The remainder of the book deals with quantal assays. Dr Finney's book on probit analysis gives a fuller treatment of this topic, but he now deals specifically with dilution assays and writes chiefly for the worker for whom bio-assay is a routine technique rather than a research tool. Probits, logits and the angular transformation are all discussed, as are many of the alternative methods which have been suggested from time to time. There is a long list of references, a very complete set of tables, several of which are new, and an index.

All statisticians and biologists who use assay techniques should possess a copy of this book. In clarity and freedom from misleading statements it surpasses its few predecessors in the field; and one can be fairly certain that, if the solution to a particular problem is not to be found in it, no solution was known at the date of going to press. Dr Finney has performed a most valuable service to workers in a field of great and growing importance.

M. J. R. HEALY

Insect Resistance in Crop Plants. By REGINALD H. PAINTER. Pp. x+520, with 67 text-figures and photographs. New York: The Macmillan Company. 1951. £3. 11s. 6d.

The great emphasis laid on the chemical control of insects makes it a refreshing experience to review a book dealing with purely biological relationships between crops and insects. This is not to deride the value of insecticides, but follows from what Prof. Painter calls 'the spectacular successes of the newer types of insecticides' which, 'have tended to obscure the fact that there are many other ways by which insects may be controlled'. It is unfortunate that the research required to elucidate these other means is so much slower than that needed to produce insecticides.

The references to the resistance of crops to insects are very scattered, and Prof. Painter has earned our gratitude by presenting a very large number in a readily available form. The literature is summarized in three chapters dealing with general aspects of the problem, and five dealing with particular crops—wheat, maize, sorghum, cotton and potatoes. The general chapters deal with 'The Mechanisms of Resistance', 'Factors that affect the Expression or the Permanence of Resistance', and methods and problems in breeding for resistance. These chapters will be of wide general interest to economic entomologists and plant breeders, and while those on particular crops are more likely to appeal to American workers, they contain much that will be of value in other parts of the world. The last chapter contains several hundred references to crops not dealt with in detail in the rest of the book.

To summarize such a large amount of work in a limited space inevitably leads to difficulties, one of the greatest of which is to maintain an easy or fluent continuity, and it is a minor criticism of Prof. Painter's book that he has not completely overcome this difficulty. This does not, however, detract from the book's usefulness as a work of reference—for which it is primarily intended.

The book is strongly bound and the quality of the paper and clarity of the type are good, but on the whole, the illustrations are not well reproduced. The high price may put it out of the range of many private buyers, but this book will be a valuable addition to many libraries.

F. H. JACOB

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STUDIES ON PLANT GROWTH-REGULATING
SUBSTANCES

VI. SIDE-CHAIN STRUCTURE IN RELATION TO GROWTH-REGULATING ACTIVITY IN THE ARYLOXYALKYLCARBOXYLIC ACIDS

By C. H. FAWCETT, DAPHNE J. OSBORNE, R. L. WAIN
AND RUTH D. WALKER

ERRATUM

Annals of Applied Biology, 39, 4

Page 530, 11 lines from bottom

flight *should read* light

carboxyl group, (b) a certain spatial relationship between the double bond and the carboxyl group. Aryloxyalkylcarboxylic acids were not investigated by these workers, but the growth-regulating activity in the pea test of compounds obtained by substituting groups into the side-chain of certain arylalkylcarboxylic acids was reported. Thus, the activity of phenylacetic, α -phenylpropionic and α -phenylisobutyric acids was rated as 2 : 6 : 0 respectively, expressed as a percentage of that shown by 3-indolylacetic acid, whilst mandelic, atrolactic and tropic acids were inactive. Koepfli *et al.* pointed out that the results did not warrant any conclusions with regard to the effect of substituents in the side-chain, and although the possibility of steric hindrance arising from the introduction of two methyl groups on the α -carbon atom might account for the inactivity of α -phenylisobutyric acid, the activity of isatinic acid conflicted with the view that an α -hydrogen atom is necessary for activity to be shown in this test by arylalkylcarboxylic acids.

The demonstration by Irvine (1938) that 2-naphthoxyacetic acid possessed

growth-regulating properties showed that the presence of an oxygen bridging group between ring and side-chain was also consistent with activity. Many such aryloxy-alkylcarboxylic acids are now known as growth substances. The effect of substituting the oxygen bridging group in 2-naphthoxyacetic acid by —S— or —NH— was studied by Walker (1948), who found these compounds inactive for setting tomatoes. Veldstra & Booij (1949), however, found that the activity in the Went pea test of *N*-2:4-dichlorophenylglycine is comparable with that of 2:4-dichlorophenoxyacetic and 1-naphthylacetic acids, and concluded that a side-chain containing —NH— can fulfil the requirements for this type of growth-regulating activity.

Veldstra (1944*b*) also investigated the effects in the Went pea test of substituting the carboxyl group of 1-naphthylacetic acid by a sulphonic acid or nitro group; 1-naphthylmethanesulphonic acid was inactive while 1-naphthylnitromethane showed a low activity, which was ascribed by Veldstra (1944*b*) to the presence of the tautomeric aci-form $C_{10}H_7-CH=NOOH$. Walker (1948) found both sodium 2-naphthoxy-methanesulphonate and -ethanesulphonate to be inactive for inducing parthenocarpic development of tomatoes.

In growth substances derived from indole and naphthalene, the position of the side-chain has been shown to be important. Thus 3-indolylacetic acid is active, whereas 2-indolylacetic acid possesses low activity (Kögl & Kostermans, 1935). Similarly, 1-naphthylacetic acid is active while 2-naphthylacetic acid possesses less activity (Zimmerman & Wilcoxon, 1935); 2-naphthoxyacetic acid is active yet 1-naphthoxyacetic acid has negligible activity (Veldstra, 1944*a*).

The activity of a number of homologues of 1-naphthylacetic acid was studied by Grace (1939), who found that the capacity to promote rooting was less in compounds with an odd number of carbon atoms in the unbranched side-chain than in those possessing an even number. A similar effect in the 3-indolylacetic, propionic and butyric acids had been reported by Thimann & Bonner (1938). Synerholm & Zimmerman (1947) showed that extension of the side-chain in ω -(2:4-dichlorophenoxy)alkylcarboxylic acids from C_2 to C_8 yielded compounds active in the tomato-leaf epinasty test only when an even number of carbon atoms was present in the chain. The suggestion was put forward that a β -oxidation mechanism might operate in the plant. By such means those acids possessing an even number of carbon atoms in the side-chain might be degraded to the active acetic derivative, whereas compounds possessing an odd number of carbon atoms would be converted to the parent phenol, inactive as a growth substance.

Investigations on growth-regulating activity in relation to chemical structure have been reviewed by Veldstra (1944*a*) and Wain (1949). In earlier studies carried out here, the effect of substitution into the side-chain of aryloxy acids was investigated. It was known that 2-naphthoxyacetic acid is highly active in inducing parthenocarpy in the tomato and that the compound obtained by substitution of a methyl group into the side-chain (α -(2-naphthoxy)propionic acid) has

similar activity. The corresponding compound with two methyl groups, viz. α -(2-naphthoxy)isobutyric acid, however, was found by Walker (1948) to be inactive for setting tomatoes, indicating that this further substitution destroyed a feature of the molecule which was essential for this type of growth-regulating activity.

A systematic study has now been made on the biological activity of certain aryloxyacetic acids and their derivatives with alkyl substituents in the side-chain. The object was to determine whether a hydrogen atom attached to the carbon atom *alpha* to the carboxyl group is necessary for growth-regulating activity. A brief account of these results has been published (Osborne & Wain 1951*a, b*).

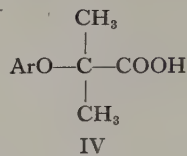
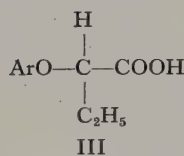
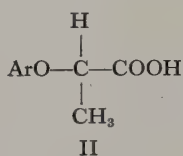
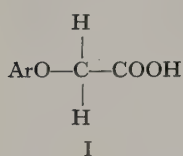
For assessing growth-regulating activity, various tests are available. It is well known, however, that certain compounds can show high activity in one test yet be weakly active in another. Thus 2-naphthoxyacetic acid shows negligible activity in the *Avena* curvature test (Avery, Berger & Shalucha, 1942), although it is highly effective in inducing parthenocarpic development in the tomato (Wain, 1950). Again, 1-naphthylacetic acid induces a small curvature in the standard *Avena* test, is active in the Went pea, tomato-leaf epinasty and rooting tests yet possesses negligible activity for producing parthenocarpic tomatoes, even at high concentrations. Other examples are cited by Thimann & Schneider (1939). A primary requisite for any growth response is that the compound should reach the site of action within the plant tissue. The penetrative properties of the growth substance molecule are therefore directly concerned, though this factor is possibly of more importance in certain types of growth response than in others (Veldstra, 1944*a*). Such considerations illustrate the difficulties inherent in comparing the growth-regulating activities of different compounds. It is therefore evident that to obtain comprehensive assessment, a compound should be examined in a wide range of tests, each depending on a different type of growth response.

In the present work seven test methods were employed, which may be grouped into two main classes: those which assess cell extension and those in which cell division is also involved. The following tests have been considered to depend on cell extension: the tomato-leaf epinasty test (Zimmerman & Hitchcock, 1937); the *Avena* cylinder elongation test (Bonner, 1933); the *Avena* curvature test (Went, 1928). The tomato parthenocarpy test (Zimmerman & Hitchcock, 1944), the tomato-leaf rooting test (Hitchcock & Zimmerman, 1938) and the ability to induce morphogenic responses in growth (Osborne & Wain, 1951*c*) have been classified as depending upon cell extension and division. Curvatures in the pea test (Went, 1934) would appear to be the result of a complex form of differential growth which cannot be satisfactorily classified in either group (Jost & Reiss, 1936).

MATERIALS AND METHODS

Thirty-two aryloxyalkylcarboxylic acids of general formulae I, II, III, IV were synthesized, making eight complete series derived from different phenols. The aryl (Ar) groups were: phenyl, 2-chlorophenyl, 4-chlorophenyl, 2:4-dichloro-

phenyl, 2:4:5-trichlorophenyl, 2-methyl-4-chlorophenyl, 1-naphthyl and 2-naphthyl:



Preparation of aryloxy acids

Aryloxyacetic, α -aryloxy-propionic and -butyric acids were prepared by condensing the sodium salt of the appropriate phenol with the ethyl ester of the monohalogen substituted fatty acid in alcoholic solution and hydrolysing the product (Synerholm & Zimmerman, 1945).

α -Aryloxyisobutyric acids were generally made by condensation of acetone, chloroform and the phenol in presence of solid sodium hydroxide (Bargellini, 1906). In certain cases the compounds were prepared also from α -bromoisobutyric ester as above. The acids, together with uncorrected melting-points and neutralization equivalents, are presented in Table 1.

Assessment of growth-regulating activity

(1) *The tomato epinasty test* (Zimmerman & Hitchcock, 1937)

A lanolin solution of the compound was applied to approximately 3 cm. of the adaxial surface of the fourth petiole from the apex, and to the side of the stem above the petiole. Active compounds induced elongation of the cells in the treated region within 24 hr. and caused the petiole to bend downwards.

The activity of each compound assessed after 24 hr is presented in Table 3, and results obtained in the 2:4:5-trichlorophenoxy series are shown in Pl. 1, fig. 1.

(2) *Avena cylinder test* (Bonner, 1933)

Oats, var. Victory, were grown on moist filter-paper in an incubator at 25° C. When seedlings were roughly 3.5 cm. high (4 days old) they were decapitated by removing the apical 3 mm. After 2 hr. a second 3 mm. section was removed and discarded. A third 3 mm. portion was then cut, the rolled up primary leaf expelled, and the cylinder of coleoptile threaded on to a fine glass rod. Each cylinder was then measured using a microscope fitted with a micrometer eyepiece. The threaded sections were floated on the surface of 100 ml. of a solution of the sodium salt of the growth substance contained in a Petri dish; control cylinders were floated on distilled water. The cylinders were remeasured after 24 hr., ten sections being used per treatment. The results obtained in these experiments were subjected to statistical analysis. The figures given in Table 2 represent the increase in elongation due to treatment, recorded as a percentage of the corresponding control, after

correction for initial irregularities in material. A qualitative estimation of all results obtained in this test is given in Table 3.

Since this work was completed Mr F. Wightman has re-examined these compounds in this test using the technique of Bentley (1950) with some of his own modifications. Bentley has stated that greater growth responses are obtained using

TABLE 1. *Aryloxy acids*

	Code letters of compounds	Melting- point ° (C.)	Neutralization equivalent	
			calculated	found
Phenoxyacetic acid	PA	99-100	152.1	151.9
α -Phenoxypropionic acid	PP	114-114.5	166.1	165.9
α -Phenoxybutyric acid	PB	80	180.1	179.9
α -Phenoxyisobutyric acid	PI	97.5	180.1	181.1
2-Chlorophenoxyacetic acid	2PA	147-148	186.5	186.1
α -(2-Chlorophenoxy)propionic acid	2PP	113.5-114.5	200.5	200.2
α -(2-Chlorophenoxy)butyric acid	2PB	81.5-82.5	214.6	213.7
α -(2-Chlorophenoxy)isobutyric acid	2PI	72-74	214.6	215.5
4-Chlorophenoxyacetic acid	4PA	157-158	186.5	184.9
α -(4-Chlorophenoxy)propionic acid	4PP	114.5-115	200.5	201.3
α -(4-Chlorophenoxy)butyric acid	4PB	77-78	214.6	213.0
α -(4-Chlorophenoxy)isobutyric acid	4PI	117-118	214.6	215.4
2:4-Dichlorophenoxyacetic acid	24PA	141	221.1	220.8
α -(2:4-Dichlorophenoxy)propionic acid	24PP	116-118	235.1	235.6
α -(2:4-Dichlorophenoxy)butyric acid	24PB	80.5-81	249.1	248.0
α -(2:4-Dichlorophenoxy)isobutyric acid	24PI	45-46	249.1	248.2
2:4:5-Trichlorophenoxyacetic acid	245PA	153-155	255.6	255.1
α -(2:4:5-Trichlorophenoxy)propionic acid	245PP	179-181	269.6	269.2
α -(2:4:5-Trichlorophenoxy)butyric acid	245PB	142	283.6	282.5
α -(2:4:5-Trichlorophenoxy)isobutyric acid	245PI	92.5	283.6	283.4
2-Methyl-4-chlorophenoxyacetic acid	MCPA	119	200.5	199.2
α -(2-Methyl-4-chlorophenoxy)propionic acid	MCPP	94	214.6	213.6
α -(2-Methyl-4-chlorophenoxy)butyric acid	MCPB	87-88	228.6	227.7
α -(2-Methyl-4-chlorophenoxy)isobutyric acid	MCPI	83-84	228.6	227.2
1-Naphthoxyacetic acid	INA	192-193.5	202.1	202.6
α -(1-Naphthoxy)propionic acid	INP	150-151	216.1	217.6
α -(1-Naphthoxy)butyric acid	INB	109.5-110.5	230.1	229.6
α -(1-Naphthoxy)isobutyric acid	INI	127	230.1	230.5
2-Naphthoxyacetic acid	2NA	155-156	202.1	203.6
α -(2-Naphthoxy)propionic acid	2NP	106-107	216.1	215.3
α -(2-Naphthoxy)butyric acid	2NB	123.5-124	230.1	228.9
α -(2-Naphthoxy)isobutyric acid	2NI	122	230.1	230.7

her procedure, and this was also found here by Wightman. Although the results obtained using these different techniques were qualitatively the same, 2:4:5-trichlorophenoxyisobutyric acid was exceptional in that it showed definite activity in Wightman's experiments. The other isobutyric acids were uniformly inactive.

(3) *The Avena curvature test* (Went, 1928)

Although this test has been widely used for assessing auxin activity, synthetic compounds do not induce pronounced curvatures. Thus Avery *et al.* (1942) have

demonstrated that 2-naphthoxyacetic acid is inactive in this test at 5 p.p.m., and 2:4-dichlorophenoxyacetic acid at this concentration only induces a 6.7° curvature.

Oats, var. Victory, were raised in the dark at 24° C. and relative humidity 75-80%, in trays of damp sand. Seedlings received intervals of illumination with

TABLE 2. *Activity of aryloxy acids in the Avena cylindrica and Went pea tests*

Code letters of compound	<i>Avena cylindrica</i> test			Went pea test		
	Molar concentrations					
	10 ⁻⁵	10 ⁻⁴	5 × 10 ⁻⁴	2.5 × 10 ⁻⁵	10 ⁻⁴	5 × 10 ⁻⁴
PA	94.2	95.9	.	0	0	0
PP	110.7	114.4	112.8***	1	1	2
PB	110.4*	113.0**	113.3***	3	4	6
PI	94.8	94.1	92.8	0	0	0
2PA	97.8	105.9*	.	0	0	1
2PP	106.4**	115.2***	.	.	2	4
2PB	105.3*	107.3***	.	.	6	5
2PI	101.1	101.0	98.0	0	0	0
4PA	.	113.1***	.	2	5	3
4PP	.	109.2**	.	.	5	4
4PB	102.0	104.4**†	.	.	6	4
4PI	98.2	99.0	95.1	0	0	0
24PA	116.3***	121.2***	97.8	4	5	6
24PP	117.0***	106.5	.	5	6	3
24PB	108.3***	104.0*	.	5	6	3
24PI	96.6	95.2	88.7	0	1	1
245PA	114.6***	107.9**	.	4	4	3
245PP	116.6***	103.3	.	4	4	3
245PB	109.0***	93.2	.	4	3	3
245PI	93.0	83.7	.	4	2	Damage
MCPA	111.9***	108.1**	.	5	4	3
MCPB	110.4***	108.8**	.	6	5	3
MCPPI	114.5***	111.1***	.	5	4	3
MCPI	97.5	95.7	.	0	1	2
INA	100.6	99.4	97.2	1	2	2
INP	100.8	103.2	100.1	0	2	2
INB	99.6	99.0	.	0	2	1
INI	99.2	97.0	91.7	0	2	1
2NA	102.0	109.2***†	94.9	1	3	4
2NP	107.5	112.6***	94.8	1	2	3
2NB	102.6	107.0***	93.3	0	3	2
2NI	95.4	98.6	92.8	0	1	2
Control	100	100	100	0	0	0

* Significant at 5 % level.

** Significant at 1 % level.

*** Significant at 0.1 % level.

† Statistical significance not shown in one test.

red light during the growing period to ensure straight growth of the coleoptile and to suppress elongation of the mesocotyl. Two decapitations were carried out with an interval of 2 hr. The agar cube containing the sodium salt of the growth substance to be tested was placed unilaterally on the stump of the decapitated coleoptile

resting against, and supported by, the drawn-up primary leaf. Curvatures were recorded 90 min. after application of the block by the usual shadowgraph technique. Full details of the method employed are described by Osborne (1950). The average

TABLE 3. *Growth-regulating activity of aryloxy acids*

Code letters of compound	Activity in tomato leaf epinasty test*	Activity in <i>Avena</i> cylinder test	Activity in <i>Avena</i> curvature test (5×10^{-4} M)	Morphogenic effects in tomato plants†	Activity in tomato parthenocarp test‡	Activity in tomato leaf rooting test	Activity in Went pea test
PA	Inactive (1-5)	Inactive	.	None	Inactive	Negligible	Inactive
PP	++ (2)	+++	4.0°	L, F, S	25-50	Negligible	++
PB	++ (2)	+++	.	L, F, S	25-50	Negligible	+++++
PI	Inactive (1-5)	Inactive	Inactive	None	Inactive	Negligible	Inactive
2PA	+ (1)	+	.	None	150	Low	+
2PP	++ (1)	+++	5.5°	R	25-50	High	+++
2PB	++++ (1)	+++	.	C, R, S	25-50	High	+++++
2PI	Inactive (1-5)	Inactive	Inactive	None	Inactive	Negligible	Inactive
4PA	++ (1)	+++	.	L, F, S	25-50	Low	++++
4PP	++ (1)	++	7.0°	None	25-50	Negligible	++++
4PB	+ (1)	++	.	None	150	Low	+++++
4PI	Inactive (1-5)	Inactive	Inactive	None	Inactive	Negligible	Inactive
24PA	++++ (1)	+++	7.0°	L, S	1-5	Medium	+++++
24PP	+++ (1)	+++	7.5°	L, S	25-50	Medium	+++++
24PB	++ (1)	+++	8.0°	L, S	25-50	Medium	+++++
24PI	Inactive (1-5)	Inactive	Inactive	L	Negligible§	Low	+
245PA	++++ (1)	+++	8.0°	R, S	5	High	++++
245PP	+++ (1)	+++	8.0°	R, S	25	High	++++
245PB	+++ (1)	+++	4.5°	R, S	25	High	+++
245PI	Inactive (1-5)	Inactive	Inactive	L, C	Negligible§	Negligible	+++
MCPA	++++ (1)	+++	11.0°	R, S	5	High	++++
MCPP	+++ (1)	+++	13.0°	R, S	25	High	+++++
MCPB	+++ (1)	+++	10.0°	R, S	25	High	++++
MCPI	Inactive (1-5)	Inactive	Inactive	L	Inactive	Negligible	+
INA	+ (2)	Inactive	.	None	250	Negligible	++
INP	+ (2)	Inactive	Inactive	L, F, C	25-50	Negligible	++
INB	+ (2)	Inactive	.	L, F, C	100-250	Negligible	+
INI	Inactive (2-5)	Inactive	Inactive	None	Inactive	Negligible	+
2NA	+++ (1)	+++	6.0°	R	25-50	High	+++
2NP	+++ (1)	+++	7.0°	R	25-50	High	+++
2NB	+ (1)	+++	Inactive	None	250	Negligible	++
2NI	Inactive (1-5)	Inactive	Inactive	None	Inactive	Negligible	+

* Percentage concentration in lanolin in parentheses.

† L=morphogenic effect shown by leaves. F=morphogenic effect shown by flowers and fruit. C=callus formation at point of contact. R=root initiation. S=stunting of growth.

‡ Effective concentrations in p.p.m.

§ Some activity shown in one test at 250 p.p.m.

|| Slight but definite activity when examined by Bentley (1950) technique (see p. 235).

curvature induced by each compound in some 18-25 test plants is listed in Table 3. Compounds inducing curvatures of less than 2.5° were considered to be inactive at the concentration tested.

(4) *Capacity to induce morphological responses*

Plants, treated in the tomato-leaf epinasty test, were grown on in the glasshouse for a period of some 6 weeks. During this time, any modification in growth, leaf formation or fruit development, or the formation of roots or callus tissue on the aerial parts resulting from the application of the growth substance became apparent. A full record of the types of modifications induced by these substances has been published (Osborne & Wain, 1951c), and a summary of these results is given in Table 3.

(5) *Tomato parthenocarpy test*

The ability of substances to stimulate the development of unfertilized tomato ovaries was made the basis of a biological test (Zimmerman & Hitchcock, 1944). Details of the procedure adopted in the present work have been published (Osborne, Wain & Walker, 1952), and a full record of the percentage parthenocarpy, average fruit weight and fruit size for each treatment is given by Osborne (1950). The results presented in Table 3 are the concentrations at which each substance has been found to induce a satisfactory set of parthenocarpic fruit. Substances failing to induce any fruit development at concentrations up to 250 p.p.m. have been classed as inactive.

(6) *Tomato-leaf rooting test* (Hitchcock & Zimmerman, 1938)

Uniform tomato-leaf cuttings, 6–7 in. in length, each being the fourth leaf down from a growing point, were employed for this test. All but the terminal three leaflets were removed leaving 3–4 in. of bare petiole. The lower $2\frac{1}{2}$ in. of each cutting was soaked for 24 hr. in an aqueous solution of the sodium salt of the growth substance under test. Control cuttings were similarly soaked in distilled water. Cuttings were subsequently washed and set out in beakers of tap water with the basal 3–4 in. immersed, and kept in the glasshouse. Final records were made when control cuttings had just started to root, usually within 6 weeks.

Complete records were made of the number and length of roots induced by each treatment, and a method of assessment based on the calculation of 'Root-Inducing Capacity' was adopted. Detailed results have been published (Osborne, 1950; Osborne *et al.* 1952). A qualitative summary of the results is given in Table 3, and the rooting induced by 2:4:5-trichlorophenoxy series is shown in Pl. 1, fig. 2.

(7) *Went pea test* (Went, 1934)

The technique adopted has been fully described by Osborne (1950). Compounds were tested at three concentrations, 5×10^{-4} , 10^{-4} and 2.5×10^{-5} M. Toxic effects generally became apparent at the highest concentration and in many cases damage was severe, the tissues becoming soft, flaccid and translucent. At the lower concentrations normal inward curvatures were obtained. Detailed results are given

in Table 2 and a qualitative assessment for each compound in Table 3. The results for the 4-chlorophenoxy- and the 2:4-dichlorophenoxy- series are shown photographically in Pl. 1, fig. 3.

DISCUSSION

The present investigations represent a logical extension of the finding here in 1948 that whereas 2-naphthoxyacetic and α -(2-naphthoxy)propionic acids are highly active for inducing parthenocarpy, the corresponding *isobutyric* acid was inactive (Walker, 1948). This indicated that a hydrogen atom on the carbon atom *alpha* to the carboxyl group might be necessary for growth-regulating activity, an idea which has now received support from the examination of similar compounds.

In all studies on the relationship between chemical structure and growth-regulating activity it is advisable to use a variety of methods of assessment (Wain, 1951). In the present work seven such methods were employed using compounds of the type aryloxyacetic (I), α -aryloxypropionic (II), α -aryloxybutyric (III) and α -aryloxyisobutyric acids (IV) (see p. 234).

All these substances possess an unsaturated ring system, and a side-chain with terminal carboxyl group and therefore fulfil the basic structural requirements for plant growth-regulating activity suggested by Koepfli *et al* (1938). Furthermore, none of the phenoxyacids studied was disubstituted in the 2:6- positions, so that our investigations were not complicated by the inactivity known to arise from such substitutions (Muir, Hansch & Gallup, 1949; Fawcett, 1951).

In tests considered to depend upon a cell elongation response, viz. the tomato-leaf epinasty, *Avena* cylinder and the *Avena* curvature tests, all the aryloxyisobutyric acids showed negligible or slight activity (Tables 2 and 3). Except for phenoxyacetic acid, however, activity was generally found in the acetic, propionic and butyric acids, though the 1-naphthoxy acids in general possessed only very weak activity for inducing cell elongation.

The complete results from these three tests (Tables 2 and 3) strongly indicate that the aryloxyisobutyric acid structure is inconsistent with this type of growth-regulating activity. It is noteworthy that in these *isobutyric* acids the carbon atom adjacent to the carboxyl group (α -carbon) carries no hydrogen atom, whereas in the acetic propionic and butyric acids at least one hydrogen atom is present (see formulae p. 234).

Let us now consider the tests in which cell division as well as cell elongation are concerned. In the tomato leaf rooting test the importance of the α -hydrogen atom is best shown in the 2:4:5-trichloro-, 2:4-dichloro- and the 2-methyl-4-chlorophenoxy series. Although all *isobutyric* acids showed negligible activity in this test, poor rooting responses were also found for other compounds; this was the case, for example, with all the phenoxy- and 1-naphthoxy- derivatives.

In the tomato parthenocarpy test inactivity was again shown by the *isobutyric* acids though, in one experiment, the 2:4-dichloro- and 2:4:5-trichlorophenoxy

compounds were rated active. All other acids investigated with the exception of phenoxyacetic acid were active.

No conclusions can be drawn regarding the importance of the α -hydrogen atom in the production of morphogenic effects in the tomato plant (Table 3). Since this is a test of long duration, chemical modification of the compound within the plant tissue might account for these results.

The Went pea test is perhaps unique in that a large surface of damaged cells is in contact with the growth substance solution, and it is known that the response is the result of a number of factors (Jost & Reiss, 1936; van Overbeek & Went, 1937). In the present study using this test the α -hydrogen effect is clearly shown in the 2-chloro- and especially the 4-chlorophenoxy- series (Pl. 1, fig. 3). Some of the *isobutyric* acids were found to be active at the higher concentrations tested, though as shown in Table 2 this activity was, in general, less than that shown by the corresponding acetic, propionic and butyric acids (Pl. 1, fig. 3). 2:4:5-trichlorophenoxy*isobutyric* acid was exceptional in that it showed high activity in this test even at low concentrations, and this result was obtained consistently with highly purified material. The reasons for this activity are obscure.

In general the results presented in this paper, however, are consistent with the view that a hydrogen atom attached to the carbon adjacent to the carboxyl group plays an important role in the plant growth-regulating activity of aryloxy acids. Our results should, however, be considered in relation to views expressed by Veldstra & Booij (1949). These workers have suggested that in the growth substance molecule hydrophilic/lipophilic balance may be an important factor in determining the extent to which activity can be shown. Although this suggestion was based on results they obtained in the Went pea test, if the concept of hydrophilic/lipophilic balance is of fundamental importance, then it would seem permissible to apply it to other tests. The low activities we have found for 2-naphthoxy-*isobutyric* and -butyric acids, for example, might well be explained by too strong a lipophilic weighting. It would seem unlikely, however, that the *isobutyric* acid in every series examined possesses the degree of lipophilic overweighting which Veldstra & Booij would consider necessary to account for the observed low activity. Thus, in the phenoxy- and 2-chlorophenoxy- series the propionic acids are more active than the acetic derivatives, and the butyric acids are even more active. This being the case, it would be reasonable to expect the corresponding *isobutyric* acids to show at least some activity. These compounds are, however, inactive (Table 3). Other instances could be cited to show that the concept of hydrophilic/lipophilic balance does not satisfactorily explain the results obtained in the present investigation. As stated above, our results are more consistent with the view that an α -hydrogen atom must be present for high activity to be shown. We have considered whether the small diameter of such hydrogen atoms (1.9 Å) might permit the side-chain to rotate freely about the nucleus to ether-oxygen bond. Consideration of molecular models shows that rotation is prevented when the α -carbon carries two methyl groups.

This, and other types of steric effect, all of fundamental importance, are being further investigated here. There remains the possibility that an α -hydrogen atom *per se* is an essential structural requirement for growth-regulating activity in the aryloxy acids. Such considerations have already proved useful in investigations on mode of action. Thus, this hydrogen atom has been regarded by Smith & Wain (1951) as essential in that it associates with a specific receptor group at a surface boundary representing the primary site of action. Rhodes & Ashworth (1952), however, consider the α -hydrogen to fulfil a vital role in permitting enolization in the side-chain of the aryloxy acid. Ring closure is then postulated with the simultaneous formation of a high-energy phosphate bond.

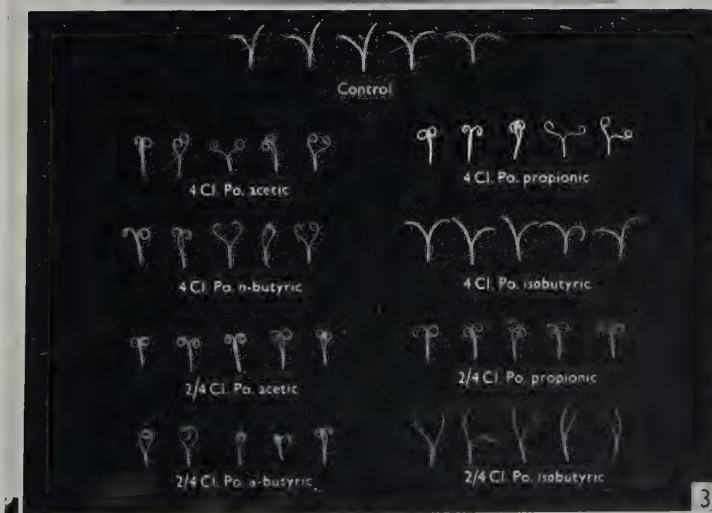
Assistance from Messrs F. Wightman, E. Maddison and D. Simons is gratefully acknowledged and thanks are also due to Prof. Bennet-Clark for providing facilities in connexion with the *Avena* curvature experiments.

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EXPLANATION OF PLATE 1

- Fig. 1. Tomato-leaf epinasty test. Left to right: control; 2:4:5-trichlorophenoxyacetic, α -(2:4:5-trichlorophenoxy)-propionic, -butyric, and -isobutyric acids, all applied as 1% solutions in lanolin.
- Fig. 2. Tomato-leaf rooting test. First row: control. Second row, left to right: α -(3-indolyl)butyric acid at 5×10^{-6} M, 2:4:5-trichlorophenoxyacetic acid at 2.5×10^{-6} M. Third row, left to right: α -(2:4:5-trichlorophenoxy)-propionic and -butyric acids at 2.5×10^{-6} M. Fourth row: α -(2:4:5-trichlorophenoxy)-isobutyric acid at 5×10^{-6} M.
- Fig. 3. Went pea curvature test. First row: control. Second row, left to right: 4-chlorophenoxyacetic and α -(4-chlorophenoxy)propionic acids. Third row, left to right: α -(4-chlorophenoxy)-butyric and -isobutyric acids. Fourth row, left to right: 2:4-dichlorophenoxyacetic and α -(2:4-dichlorophenoxy)propionic acids. Fifth row, left to right: α -(2:4-dichlorophenoxy)-butyric and -isobutyric acids. All compounds were tested at 5 p.p.m.

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STUDIES ON PLANT GROWTH-REGULATING SUBSTANCES

VII. GROWTH-PROMOTING ACTIVITY IN THE CHLORO-PHENOXYACETIC ACIDS

By R. L. WAIN AND F. WIGHTMAN

(Wye College, University of London)

(With Plate 2 and 3 Text figures)

The physiological activity of all the mono-, di- and trichloro-phenoxyacetic acids has been investigated using the pea curvature and *Avena* cylinder tests. The highest activity was shown by the 4-chloro-, 2:4-dichloro- and 2:4:5-trichloro- acids, though the 2- and 3-chloro, 2:3-, 2:5- and 3:4-dichloro- and the 2:3:4-trichloro- derivatives were appreciably active in both tests. Significant activity was also shown in either one or both tests by the 2:6-dichloro-, 2:3:5-, 2:3:6- and 3:4:5-trichloro- acids.

The activity of such compounds is considered in relation to two recent theories which attempt to relate capacity to induce a growth response with the position of substituents in the aromatic ring. It is concluded that neither theory can satisfactorily explain the relative activities of these compounds for all types of growth response.

INTRODUCTION

Since the first demonstration that 2-naphthoxyacetic acid possessed growth-regulating properties (Irvine, 1938), a number of highly active plant growth substances have been found amongst the aryloxyalkylcarboxylic acids. Some of these, in particular certain chlorinated phenoxy acids, such as 2:4-dichloro-, 2-methyl-4-chloro, and 2:4:5-trichloro-phenoxyacetic acids, have been developed for selective weed control (for recent reviews of literature see, for example, Norman, Minarik & Weintraub 1950; Templeman & Halliday, 1951). Although much other work has been carried out with various compounds of this type (e.g. see Mitchell & Marth, 1950), only recently (Leaper & Bishop, 1951) has a systematic study been made of the relative physiological activities of all the various mono-, di- and trichloro-phenoxyacetic acids. For this investigation, Leaper & Bishop prepared all fifteen acids in a pure state and examined their activity in the tomato-leaf epinasty test of Zimmerman & Hitchcock (1942), and for their capacity to initiate rooting on intact tomato stems. In addition, a study was made on their effect in inhibiting the growth of roots of *Lupinus albus* seedlings, using the method described by Macht & Livingston (1922). Although the results obtained by these test methods were in agreement, many examples are known where the activity of a compound may vary considerably with the test employed (Wain, 1951; Fawcett, Osborne, Wain & Walker, 1953). For such reasons, we have found it desirable to

use various methods of assessment when comparing the growth-regulating activity of different compounds.

Through the generosity of Dr Leaper, who made all his compounds available to us, it has been possible to examine the series further and obtain critical data on the relative activities of all fifteen acids in the pea curvature and *Avena* cylinder tests. Many of these compounds had already been synthesized and examined in this laboratory (e.g. Osborne & Wain, 1950; Osborne, Wain & Walker, 1951).

Our results for the mono-, di- and trichloro-phenoxyacetic acids, in the above two tests, are presented in this paper and briefly considered with reference to suggestions made by other workers concerning ring substitution in relation to the growth-regulating activity of phenoxy acids (e.g. see Hansch, Muir & Metzenburg, 1951; Muir, Hansch & Gallup, 1949; Leaper & Bishop, 1951; Thimann, 1952).

MATERIALS AND METHODS

Full details of the syntheses and properties of the acids used in this investigation are given by Leaper & Bishop (1951).

All compounds were examined as solutions of their sodium salts at the stated concentration of the acid.

The pea curvature test (Went, 1934). The experimental procedure adopted was essentially that of Went & Thimann (1937) with some modifications in technique (Smith, Wain & Wightman, 1952). In particular, it was found that unless the pea seedlings were exposed to red light during growth, the stem material showed only low sensitivity to growth-substance treatment. This importance of the red light factor during growth has recently been emphasized by Kent & Gortner (1951).

The Avena cylinder test (Bonner, 1933). The technique developed by Bentley (1950) and further modified by Smith *et al.* (1952) was adopted as the standard procedure.

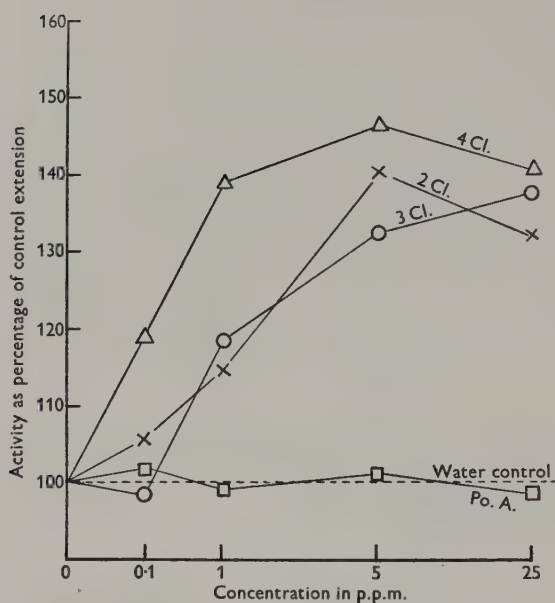
EXPERIMENTAL RESULTS

The activities of all the mono-, di- and trichloro-phenoxyacetic acids in the pea curvature test are shown in Pl. 2, figs. 1-3. The results obtained in the *Avena* cylinder test are presented graphically in Text-figs. 1-3. Phenoxyacetic acid itself has been included in both tests. The trichloro- acids were not tested in the pea test at 100 p.p.m., because at this concentration phytotoxic effects were usually apparent.

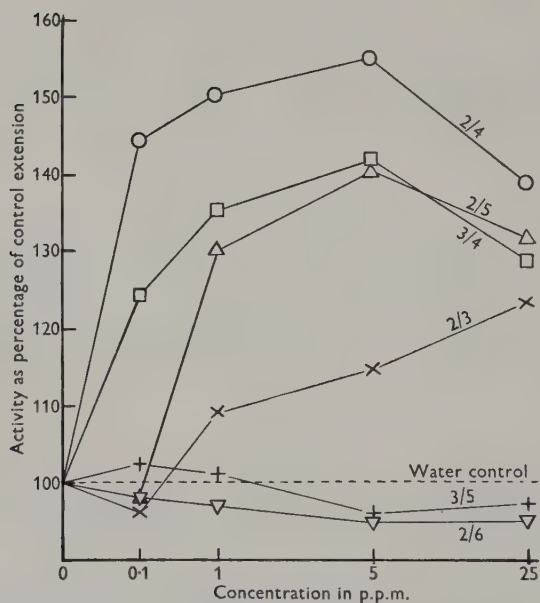
DISCUSSION

The results obtained in the present investigation show similar relative activities for the various chlorophenoxy acids in the pea curvature and *Avena* cylinder tests. Further, the results correlate well with those reported by Leaper & Bishop (1951) using other test methods.

In the cylinder test, the 4-chloro-, 2:4-dichloro- and 2:4:5-trichloro- acids are more active than any of their respective isomers at all concentrations (Text-figs 1-3).

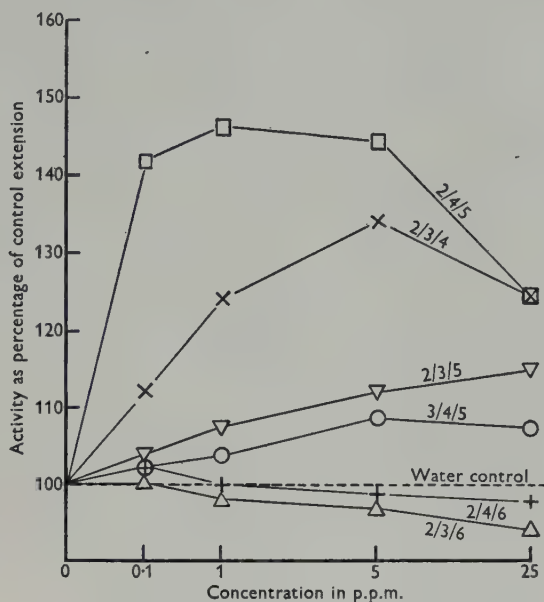


Text-fig. 1. Relative activity of phenoxyacetic acid and the monochloro-phenoxyacetic acids in the *Avena* cylinder test.



Text-fig. 2. Relative activities of the dichloro-phenoxyacetic acids in the *Avena* cylinder test.

The 2:4- and 2:4:5- acids, however, are clearly more active than the 4-chloro-derivative at low concentrations. In this test over the whole concentration range, 2:4-dichloro-phenoxyacetic acid is the most active of all compounds studied. The results obtained in the pea test again reveal high activity for the above three acids (Pl. 2, figs 1-3).



Text-fig. 3. Relative activities of the trichloro-phenoxyacetic acids in the *Avena* cylinder test.

The importance of studying the activity of a compound over a range of concentrations is well demonstrated in the present results, both for the pea curvature and *Avena* cylinder tests (see Pl. 2 and Text-figs. 1-3). Thus, for example, at 0.1 p.p.m. the 2-chloro- acid possesses negligible activity in each test, whereas the 2:4- and 2:4:5- acids are highly active at this concentration. At 5 p.p.m., however, the relative activities of the three acids are not appreciably different.

Reference to Pl. 2 and Text-figs. 1-3 shows that in addition to the three acids discussed above, the following compounds show appreciable activity in both tests: 2-chloro-, 3-chloro-, 2:3-dichloro-, 2:5-dichloro-, 3:4-dichloro- and 2:3:4-trichloro-phenoxyacetic acids. Activity in all these compounds is consistent with the view put forward by Muir and co-workers (Muir *et al.* 1949; Muir & Hansch, 1951; Hansch *et al.* 1951), that capacity to induce a growth response is associated with the presence of at least one unsubstituted *ortho* position in the aromatic ring. Further, seven of these active compounds possess two unsubstituted positions in the ring *para* to each other, a requirement which Leaper & Bishop (1951) consider to be associated with growth-regulating activity. The fact remains, however, that a

number of compounds have been shown to possess significant activity which, on the basis of the above two hypotheses, should be inactive. For the theory of Muir *et al.*, no exceptions of this kind are apparent in the *Avena* cylinder test. In the pea test, however, the 2:6-dichloro- and the 2:3:6-trichloro- acids are active and the 2:4:6- derivative shows slight activity. Thimann (1952) has reported similar findings for the 2:6- and 2:4:6- acids. This activity of the 2:6- acid was not found in a previous investigation (Seeley & Wain, 1950), in which the test material was grown in absence of red light and did not possess maximum sensitivity to growth substance treatment (Kent & Gortner, 1951).

Considering our results in relation to the theory put forward by Leaper & Bishop, certain inconsistencies are again apparent. Thus, in the cylinder test, the 2:3-, 2:3:4-, 2:3:5- and 3:4:5- acids, all compounds not possessing two unsubstituted positions in the aromatic ring *para* to one another, are active. Again, in the pea test, activity is shown by these compounds as well as by the 2:6-, 2:3:6- and 2:4:6- derivatives.

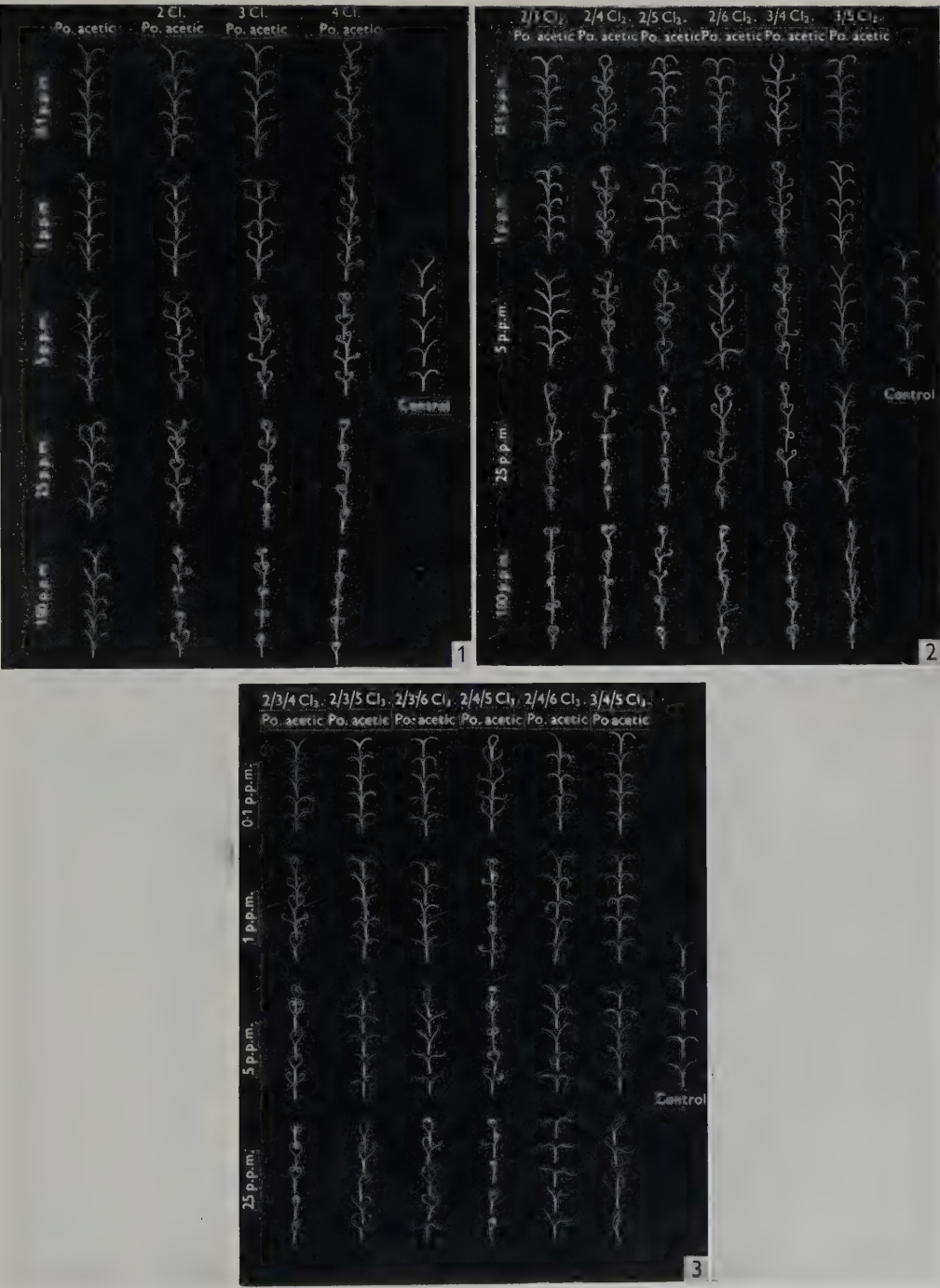
It is clear, therefore, from the present results, that neither theory discussed above will satisfactorily explain the growth-regulating activity exhibited by the chloro-phenoxyacetic acids in the *Avena* cylinder and pea curvature tests. It is of interest here to note that Thimann (1952) has also found neither theory adequate to account for the activity of certain of these acids in the pea test.

The authors gratefully acknowledge the assistance of Mrs E. Lawson in the biological testing and of Mr D. Simons for photographs. We are also greatly indebted to Dr J. M. F. Leaper for samples of the fifteen chloro-phenoxyacetic acids.

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EXPLANATION OF PLATE 2

Fig. 1. Relative activity of phenoxyacetic acid and the monochloro-phenoxyacetic acids in the pea curvature test.

Fig. 2. Relative activities of the dichloro-phenoxyacetic acids in the pea curvature test.

Fig. 3. Relative activities of the trichloro-phenoxyacetic acids in the pea curvature test.

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EXPERIMENTS WITH ROOT CUTTINGS OF BRUSSELS SPROUT

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(With 3 Text-figures)

A technique for the propagation of Brussels sprout by means of root cuttings is described. Adventitious shoots arise exogenously on callus tissue which develops around the base of side roots. Cuttings sometimes rot without forming adventitious shoots, and cuttings which remain sound do not all produce shoots. Rotting may largely be prevented by planting cuttings with the proximal end exposed above the surface of the medium, and by allowing the root portions to dry before planting. Surface sterilization with mercuric chloride controls rotting but reduces bud formation. Individual plants differ in their capacity to form buds on root cuttings, and this difference is carried by the clones derived from them. Portions of root form more buds if cut into several pieces than if planted intact.

INTRODUCTION

Several workers, in particular Wellensiek (1948) and Snee (1948), have drawn attention to the role that vegetative propagation might play in the maintenance and improvement of cultivars of *Brassica oleracea*. One of the most promising methods for clonal propagation of this species is by means of root cuttings, a method which was first described by Isbell (1945) and later by Snee (1948) and North (1952).

First attempts by the writer to use root portions for the propagation of Brussels sprout gave poor results, since many cuttings rotted without producing adventitious shoots, even when all reasonable precautions had been taken to maintain hygienic conditions, and not all the cuttings which remained sound produced adventitious shoots. This paper describes the results of some experiments designed to find solutions to these two problems.

OUTLINE OF TECHNIQUE

Plants of Brussels sprout (Ashwell's strain) bearing fully developed 'sprouts' were lifted during the autumn. Roots of diameter 5–10 mm. were severed as close to the main stem as possible, laid on a coarse-mesh sieve and washed free of soil by a strong jet of water. Unless otherwise stated, the cleaned roots were cut into lengths of 6 cm., and only the proximal portions of the roots were used as cuttings. Thin side roots were carefully severed from the prepared root portions.

Five-inch diameter flower-pots containing a coarse quartz sand were sterilized in the autoclave. Cuttings were planted vertically in the sand with the proximal cut

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surface uppermost. Ten cuttings were spaced as evenly as possible in each flower-pot in such a manner that they formed a ring, concentric with, and about 1.5 cm. from, the inside of the walls of the pot. Equal numbers of cuttings from individual plants were distributed throughout the different treatments in each experiment.

Pots of root cuttings were placed in a greenhouse (temp. 60–70° F.) and watered overhead daily with fresh tap water.

Six to eight weeks after isolation of the root portions the adventitious shoots were removed, and planted as stem cuttings in a sterile mixture of half quartz sand and half Vermiculite. They were kept in a propagating case for about 10 days, after which time they had generally formed roots and were transplanted to 3 in. flower-pots.

DEVELOPMENT OF THE ROOT CUTTINGS

Brussels sprout roots are generally diarch but not infrequently triarch. Roots selected for cuttings have two or three fairly well-marked longitudinal rows of thin side roots which arise through a series of weak transverse ridges in the cortex (Fig. 1, *A*).

A few days after the cuttings have been planted the transverse ridges become more pronounced, and within 5–6 days the hypodermis ruptures along the crest of these ridges revealing unprotected callus tissue beneath. The breaks in the hypodermis are, as a rule, roughly lenticular in shape (Fig. 1, *B*).

At this stage new roots commence to appear. Most of these push their way through the tissue beneath the breaks in the hypodermis, but wound roots are sometimes produced at the distal end of the cutting (Fig. 1, *C*).

Adventitious buds arise exogenously on the callus tissue which develops in the area associated with the breaks in the hypodermis (Fig. 1, *D*); they have very rarely been observed to form on wound callus. The majority of the buds develop on the proximal or exposed end of the cutting, but shoots occasionally form below the sand and push their way to the surface.

Some cuttings begin to develop buds 9 days after planting, but by 40–45 days after isolation bud production generally ceases (Fig. 2), and those cuttings which have not formed buds by this time as a rule fail to do so, even if they remain sound. As many as fifty buds may be formed on a cutting 6 cm. long, but the average number is in the neighbourhood of ten per cutting. As a rule no more than four buds on each cutting grow into shoots (Fig. 1, *F*); the remainder stay dormant unless some of the shoot growth is removed. Adventitious shoots often form roots close to their place of origin on the cutting.

Some cuttings develop more or less spherical masses of green callus tissue in place of normal adventitious buds (Fig. 1, *E*). Similar callus masses may also arise from the pericycle at the proximal cut surface of the root portion. Barren callus tissue of this nature, and that bearing adventitious buds, eventually becomes suberized.

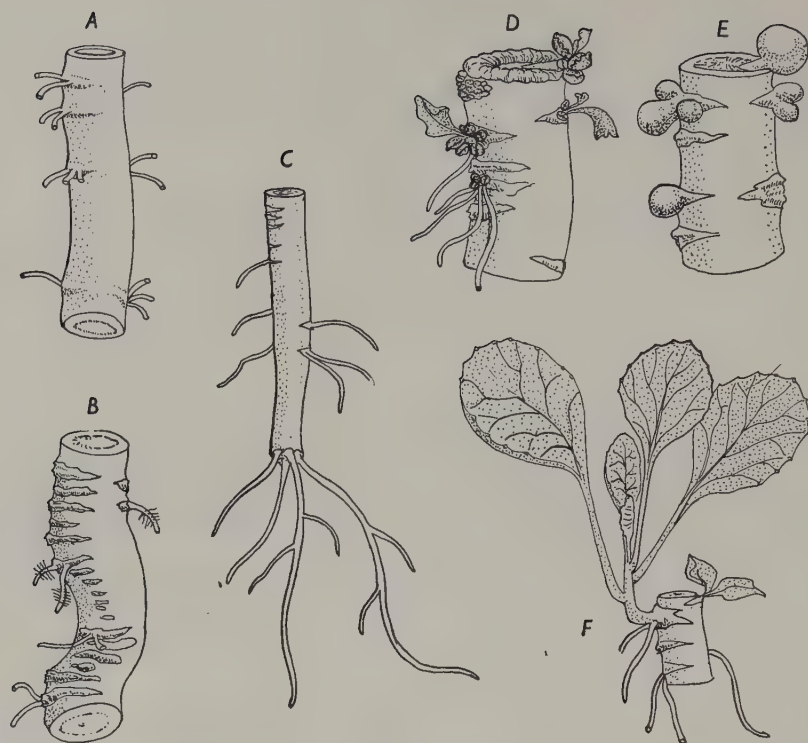


Fig. 1. Brussels sprout root cuttings. *A*, freshly isolated root portion; *B*, 5 days after isolation; *C*, cutting showing development of wound roots at distal end; *D*, adventitious buds forming on proximal end of cutting; *E*, callus masses developing in place of adventitious buds; *F*, shoot development 6 weeks after isolation of root portion.

EXPERIMENTS ON THE CONTROL OF ROTTING

Exp. 1. Depth of planting

Cuttings were prepared and planted on 20 October with the proximal cut surfaces 2.5 cm., 1 cm., 1 mm. above and 1 mm., 1 cm., 2.5 cm. below the surface of the sand. Two pots of cuttings were prepared for each treatment.

Fifty-four days after planting the number of cuttings which had rotted entirely, the number of cuttings with shoots, and the fresh weight of shoot growth was recorded (see Table 1).

Cuttings planted with the proximal cut surface 1 mm. above the level of the sand, and those completely buried, all rotted. Fewer cuttings rotted, and more produced shoots, when the uppermost cut surface was raised 2.5 cm. above the sand than if it were only raised 1 cm. (χ^2 for the difference in these results was significant for $P=0.05$.) An exposure of 2.5 cm. resulted in an average of 0.68 g. of shoot growth per cutting with shoots, whereas an exposure of 1 cm. resulted in a shoot growth of only 0.30 g. per cutting with shoots.

TABLE I. *Shoot production and rotting of cuttings planted at different depths*

Twenty cuttings used for each treatment.

Position of proximal cut surface of cutting in relation to sand	Above			Below		
	2.5 cm.	1 cm.	1 mm.	1 mm.	1 cm.	2.5 cm.
No. of cuttings rotted	3	11	20	20	20	20
No. of cuttings with shoots	16	6	0	0	0	0
Fresh wt. of shoots (g.)	10.86	1.79	0	0	0	0

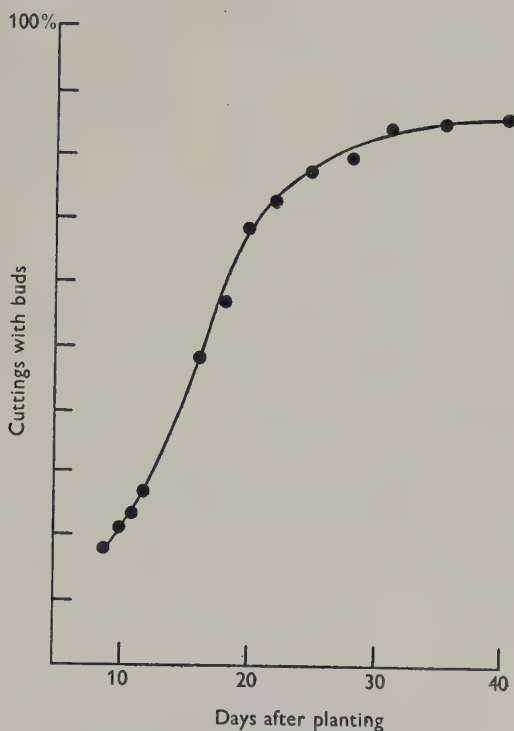


Fig. 2. Rate of bud production.

Exp. 2. Drying of cuttings before planting

On 30 October, 160 cuttings were prepared. Forty were planted as a control shortly after preparation, forty were soaked in tap water for 48 hr. at 60–70° F., forty were dried for 16 hr., forty were dried for 24 hr. Drying was effected by laying the cuttings on a clean sheet of paper in the greenhouse where the relative humidity, as measured by a hair hygrograph, remained fairly constant at 75% for 16 hr. Cuttings which were to be dried for 24 hr. were removed to the laboratory after 16 hr. treatment, as the onset of rain had introduced the risk of water dripping on to the roots. The relative humidity of the laboratory remained fairly constant at 60%.

Before and after drying, the cuttings were weighed. Twenty-eight days after planting the number of entirely rotted cuttings was recorded, and 50 days after planting the number of entirely rotted cuttings was again recorded, together with the number of cuttings with shoots and the fresh weight of shoots (Table 2).

TABLE 2. *Shoot production and rotting of cuttings dried and soaked before planting*

Forty cuttings used for each treatment.					
	Control	Soaked 48 hr.	Dried 24 hr.	Dried 16 hr.	Sig. diff. ($P=0.05$)
% loss of wt. on drying			38.6	29.1	
No. rotted (28 days):					
Actual	5	15	3	0	
Angular transformation	20.7	37.8	15.8	0	12.6
No. rotted (50 days):					
Actual	30	29	24	16	
Angular transformation	60.0	60.0	50.8	39.2	12.6
No. with shoots (50 days):					
Actual	5	4	9	18	
Angular transformation	20.7	18.4	28.3	42.1	12.6
Fresh wt. of shoots (g.) (50 days)	1.12	2.31	6.33	12.24	8.09

Considerable shrinkage of the cuttings took place after 16 hr. drying, and cuttings dried for 24 hr. were markedly shrivelled.

Drying for 16 hr. gave a significant reduction in the amount of rotting and a significant increase in shoot growth as compared with the control and soaking treatment. Drying for 24 hr. showed similar, although less marked, trends, but the results were not significant. Cuttings which had been soaked commenced to rot before those of the control, but 50 days after planting no more of the soaked cuttings had rotted than those of the control.

Exp. 3. Effect of some surface sterilants

On 19 October, 200 root portions 7 cm. long were subjected to treatment for 30 min. in: tap water; 1/1000 mercuric chloride; 1/2000 mercuric chloride; 1/1000 silver nitrate; 1/20 Chlorox (a proprietary disinfectant of the bleaching-powder type) respectively. All root portions, except those treated in Chlorox, were washed in tap water after treatment. The thin side roots were removed after treatment and the root portions were trimmed to 6 cm. lengths. The prepared cuttings were planted with the uppermost cut surface 1 cm. above the sand.

Twenty-five days after planting the number of dead cuttings was recorded, and 60 days after planting the figure was reassessed and the fresh weight of shoots was recorded (Table 3).

Both treatments with mercuric chloride gave a significant decrease in the number of cuttings which had rotted by 60 days. Silver nitrate damaged the cuttings, as is evidenced by the number of dead cuttings recorded 25 days after

planting. Chloros did not appear to damage the cuttings, or to give any control over rotting. The differences in fresh weight of shoots were not significant.

TABLE 3. *Surface sterilization of cuttings for 30 min.*

Forty cuttings used for each treatment.

	Tap water	HgCl ₂ 1/1000	HgCl ₂ 1/2000	AgNO ₃ 1/1000	Chloros 1/20	Sig. diff. (<i>P</i> =0.05)
No. dead (25 days)						
Actual	0	0	1	12	0	
No. dead (60 days):						
Actual	23	5	6	27	20	
Angular transformation	49.3	20.7	22.8	55.3	45	12.6
No. with shoots (60 days):						
Actual	13	20	19	13	14	
Angular transformation	34.7	45.0	43.6	34.7	36.3	12.6
Fresh wt. of shoots (g.) 60 days	7.80	19.46	17.20	15.77	12.23	N.S.

Exp. 4. Surface sterilization with mercuric chloride for different periods of time

On 20 December, 250 root portions 7 cm. long were subjected to treatment in 1/1000 mercuric chloride for 0, 5, 10, 30 and 60 min. After treatment they were washed in tap water and prepared and planted as in Exp. 3.

Fifty-five days after planting the number of dead cuttings, number of cuttings with shoots, and the weight of shoots were recorded (Table 4).

TABLE 4. *Surface sterilization of cuttings with mercuric chloride for different periods of time*

Fifty cuttings used for each treatment.

	Control	5 (min.)	10 (min.)	30 (min.)	60 (min.)
No. dead (55 days)	5	0	0	1	0
No. with shoots (55 days)	30	27	21	23	19
Fresh wt. of shoots (g.) (55 days)	31.4	28.3	21.9	16.8	15.2

Five of the control cuttings and only one of the 200 treated cuttings rotted. The number of cuttings producing shoots and the fresh weight of shoots were both reduced by treatment for only 5 min., and longer treatment times caused greater reductions.

EXPERIMENTS ON THE REGENERATIVE CAPACITY OF CUTTINGS

Exp. 5. Difference in regenerative capacity of individual plants

Fifteen roots from each of nine plants raised from seed sown 9 February 1950 were collected on 12 January 1951. Cuttings 6 cm. long were prepared from the proximal end of these roots and planted in fifteen pots with the uppermost cut surface projecting 1.5 cm. above the surface of the sand. They were arranged so that each pot contained one cutting from each plant. Immediately after planting, the diameter of the upper cut surface of each cutting was measured to the nearest millimetre.

The number of buds or shoots visible on each cutting was recorded 40 and 70 days after planting (Table 5).

TABLE 5. *Difference in regenerative capacity of individual plants*

	Fifteen cuttings obtained from each plant.									Sig. diff. ($P=0.05$)
	I	II	III	IV	V	VI	VII	VIII	IX	
Mean top diameter. (mm.)	6.8	7.6	6.1	7.1	7.7	6.9	8.3	8.3	7.9	—
Range of top diam. (mm.)	5-9	5-11	5-7	5-9	6-9	6-8	7-11	6-11	6-10	—
Cuttings with buds (40 days):										
Actual %	27	67	93	93	100	100	100	100	100	
Angular transformation	31.3	54.9	74.7	74.7	90.0	90.0	90.0	90.0	90.0	20.5
Mean no. of buds per cutting with buds (40 days)	1.50	3.20	4.79	5.71	8.80	11.50	14.47	14.53	16.60	—
Mean no. of shoots per cutting with shoots (70 days)	1.00	1.90	1.64	1.71	3.07	3.40	3.20	3.33	3.33	—

There was a significant difference between the percentage of cuttings from plants I and II which produced buds, and also between plants II and V-IX. The differences in bud-producing capacity between plants were not significantly correlated with differences in the thickness of the cuttings.

Not all the buds developed into shoots, but the correlation between the mean number of buds per budded cutting at 40 days and shoots per cutting with shoots at 70 days, $+0.918$, was significant ($P=0.01$).

Exp. 6. Regenerative capacity of clones

Stem cuttings were taken on 24 March 1951 from shoots formed by some of the root cuttings used in the previous experiment. The ramets thus derived were planted, 3 ft. apart each way, in the field on 25 April 1951. They subsequently developed into apparently normal plants.

On 21 January 1952 forty root cuttings from ramets of each of plants I, II, III, IV, V and IX were planted. As a subsidiary observation the number of fine side roots was recorded on a scale 0 (no small branch roots) to 5 (a large number of small branch roots).

Forty-six days after planting, the number of cuttings with buds, and the number of buds per cutting, were recorded. In Table 6 the results of this experiment are compared with those obtained from roots of the original plants in 1950.

There was a significant difference between the percentage of cuttings from clones I, II and the remainder which formed buds, in 1951, as there was with the original plants in 1950. There appears to be a correlation between the number of buds formed by cuttings from the original plants in 1950 and by cuttings from ramets derived from them in 1951. Ramets from V and IX produced fewer buds

than was expected; however, they formed more buds than those from clones I and II, which is similar to the results obtained from the original plants in 1950.

There was no apparent correlation between amount of root branching and regenerative capacity of the ramets.

TABLE 6. *Regenerative capacity of ramets compared with that of seedling plants from which they were derived*

	(1) Fifteen cuttings obtained from each plant. (2) Twenty cuttings obtained from each plant.						Sig. diff. ($P=0.05$)
	I	II	III	IV	V	IX	
<i>Original plant:</i>							
Cuttings producing buds (40 days) % (1)	27	67	93	100	100	100	—
Angular transformation	31.3	54.9	74.7	90.0	90.0	90.0	20.5
No. of buds per cutting with buds (40 days)	1.50	3.20	4.79	5.71	8.80	16.60	—
<i>Clone plant:</i>							
Cuttings producing buds (46 days) % (2)	10	20	87	67	53	87	—
Angular transformation	18.4	26.6	68.9	54.9	46.7	68.9	14.5
No. of buds per cutting with buds (46 days)	1.00	1.50	5.38	5.80	3.37	3.96	—
Root branching	2	5	3	4	2	3	

Exp. 7. Regenerative capacity of different portions of root

On 12 January, eighty root portions, each 12 cm. long, from ramets of a single clone were prepared as for cuttings. After washing they were allowed to dry overnight, and the following day twenty cuttings of portions were prepared as illustrated in Fig. 3, and planted with the uppermost cut surface projecting 2 cm. above the sand.

Forty-seven days after planting, the number of cuttings with buds, and the number of buds per cutting, were recorded. Sixty days after planting the number of cuttings with shoots, the fresh weight of shoots, and the number of rotted cuttings were recorded (Table 7).

Comparing the most proximal portions of roots, there was no evidence that shorter cuttings are less likely to form shoots, or to produce fewer buds per cutting, than longer cuttings. The roots showed a distinct but weak polarity in their capacity for bud production, but this effect may have been due, at least in part, to differences in thickness of the root portions. There was a distinct positive correlation between the number of portions into which a 12 cm. length of root had been divided, and the total number of buds formed by the original length of root. There was no significant difference between the weight of shoot growth formed by a 12 cm. length of root whether it were divided into two, three or four pieces or left intact.

TABLE 7. *Regenerative capacity of different portions of root*

Twenty cuttings used for each treatment.

	Portion (see Fig. 3.)									
	A ₁	B ₁	B ₂	C ₁	C ₂	C ₃	D ₁	D ₂	D ₃	D ₄
Length of proximal portion (cm.)	12	6	—	4	—	—	3	—	—	—
No. cuttings with buds (47 days)	12	11	11	13	11	16	15	12	11	8
	12	22		30			46			
No. buds per cutting with buds (47 days)	53	42	33	67	32	27	51	36	35	15
	53	75		126			137			
Fresh wt. of shoots (60 days) (g.)	14.89	10.25	3.30	7.09	3.03	2.53	6.10	3.99	2.76	1.66
	14.89	13.55		12.65			14.51			

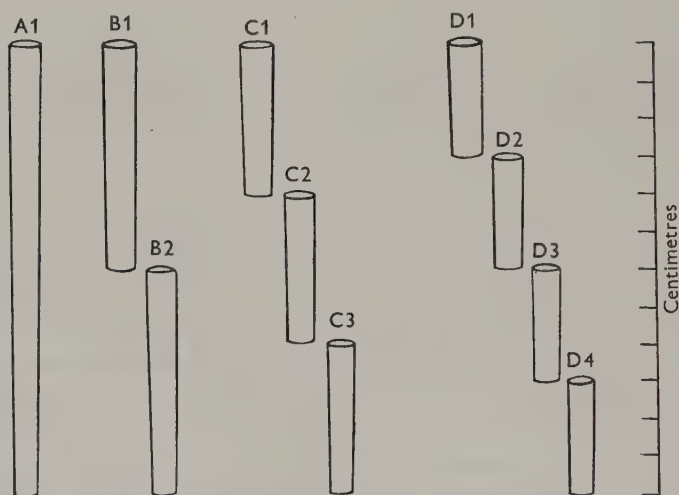


Fig. 3. Root portions used in experiment 7.

DISCUSSION

The results of Exp. 1 are similar to those obtained by Upshall (1936), who found that root cuttings of apple and plum gave better shoot growth, and rotted less, when planted with the upper cut surface 0.6–2.5 cm. above the medium than when it was buried 0.6 cm. below the medium. These results also support Snee's (1948) recommendation that cabbage root cuttings should be planted with 1 cm. projecting above the surface of the medium, but they indicate that an exposure of 2 cm. is likely to give better results than 1 cm.

It may be argued that light conditions, aeration, or the moisture relationship of the exposed portion of the cutting, or a combined effect of two or more of these

factors, determine the degree of control of rotting and apparent stimulation of shoot production. A full examination of the influence of these factors has not been made, but the results obtained in Exp. 2 show that moisture relationships play an important part in the control of rotting of Brussels sprout root cuttings.

Desiccation may control rotting, partly by killing pathogenic organisms on the exterior of the cutting, and perhaps partly by altering the physiological condition of the roots—making them more resistant to pathogenic attack and at the same time stimulating shoot production.

Almost complete control over rotting can be obtained by surface sterilization; this suggests that death of the cuttings is caused by entry of pathogens from outside rather than by breakdown of tissues. It is not surprising that Brussels sprout root cuttings are particularly susceptible to damage by pathogens, for, within a few days after planting, the tissue swells around the origin of the numerous branch roots and presents masses of unprotected callus tissue that presumably allows easy entry to disease organisms. This behaviour of roots, recorded for a wide range of species, has been discussed by Priestly & Swingle (1929) who comment upon the vulnerability of the 'callus cushions' to the entry of pathogens.

Surface sterilization with mercuric chloride gives protection to the cuttings, but inhibits bud production.

The variation in regenerative capacity of Brussels sprout root cuttings appears to be determined primarily by differences in the inherent regenerative capacity of the plants from which they were derived. This could be due to variation in genetical constitution, infection with virus, or a combination of both these factors. Clone plants used in Exp. 6 all appeared to be infected with cabbage mosaic virus, and yet some roots from each clone produced adventitious buds. Unfortunately, the infection was not confirmed, but these observations suggest that infection with virus does not prevent bud formation, although it may play some role in the limitation of bud production.

It is well known that species differ in their capacity for regeneration by adventitious buds. Hagemann (1931) found that only twelve out of forty-seven species of crucifers were capable of forming adventitious buds on leaf cuttings. Closely related varieties of some species have also been recorded as differing in their capacity for forming adventitious buds; Prevot (1939), for example, has shown that the capacity of cultivars of *Begonia rex* to form buds on isolated leaves is a dominant hereditary character. Individual Brussels sprout plants within a strain vary considerably in their genetical constitution, both in respect of morphological and physiological characters, and it seems reasonable to assume that their capacity for regeneration by root cuttings is also an inherited character.

The results of Exp. 7, showing that long roots produced more buds when cut into pieces than when left intact, indicate that the number of buds which may form on a cutting is limited. This limitation is probably brought about by the earliest formed buds inhibiting the differentiation of others through the agency of growth sub-

stances. Stoughton & Plant (1938) have shown that the formation of adventitious buds by root cuttings of *Crambe maritima* is favoured by low concentrations, and of roots by high concentrations, of growth substances. Similarly, Howard (1940) found that the differentiation of shoots or roots on decapitated seedlings of kale (*Brassica oleracea*) is governed by auxin concentration. The observation that adventitious shoots formed on Brussels sprout root cuttings sometimes develop roots close to their place of origin on the cutting, indicates that they are probably very effective auxin producers.

The regenerative capacity of Brussels sprout root cuttings is probably governed by the concentration of growth substances in the root portion at the time of isolation, and the amount of growth substances produced by the earliest formed buds. This hypothesis would not preclude an acceptance of the theory that regenerative capacity is an inherited character, for the auxin-producing capacity of individual plants, and their reaction to a given concentration of auxin may be governed genetically.

The fact that in Exp. 7 no significant difference was found between the weight of shoot growth formed by a 12 cm. length of root, whether it was divided or left intact, suggests that in sterile sand the amount of shoot growth is determined by the amount of food reserves in the cutting.

Several hundreds of ramets raised by the method described in this paper have been planted in the field; they have developed into apparently normal plants forming 'sprouts' and later flowers and seed. It is anticipated that, although the method could easily be applied by market growers, the cost of raising clone plants would preclude their use for the production of market crops of 'sprouts': the method is primarily intended to produce seed-bearing clone plants for various stages in the maintenance and improvement of seed stocks and for experimental work.

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A STUDY OF THE EPIDEMIOLOGY OF CLUB-ROOT DISEASE OF BRASSICAE

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(With 3 Text-figures)

The epidemiology of club-root disease of cabbages has been studied by growing plants in naturally and artificially contaminated soils under varying conditions of moisture, temperature, spore load, soil reaction and lime content.

The disease can occur in both acid and alkaline soils. By attention to the interaction of factors influencing infection the presence or absence of an attack, as well as its extent and severity, can be predetermined.

In acid soils, very favourable conditions for the incidence of the disease are a moisture content equal to about 70% of the maximum water-holding capacity and a mean air temperature of 18–23° C. Fluctuations in soil moisture at high levels provide as favourable conditions for infection as a high constant moisture. The level of spore load (within the limits of 10^3 to 2.5×10^7 spores per g. of soil) does not influence the number of diseased plants in acid soils when favourable conditions for an attack and for good growth of the host are provided. Under less favourable conditions spore load does exercise an effect.

The disease occurred in both naturally and artificially contaminated soils showing a pH of 7.8 at planting time; in the former, the pH was adjusted to 7.8 by liming in the field over a period of years, and in the latter by treatment with pure calcium hydroxide shortly before planting. In alkaline soils infection is favoured by high moisture content, high temperature and high spore load. Satisfactory conditions for infection are provided, as in acid soils, at a moisture content of 70% of the maximum water-holding capacity, it being immaterial whether this level is constantly maintained or fluctuating at high levels. No diseased plants occurred when the soil moisture fluctuated or remained constant at low levels. Severe attacks occurred in alkaline soils when the air temperature fluctuated between points much higher and lower than the mean temperature of 23° C. It is suggested that the optimum temperature for infection is higher than 23° C. In alkaline soils at moisture contents and temperatures favourable for infection a heavy attack can develop only in the presence of a high spore load.

In general, it was found that the conditions which were most favourable for the incidence of the disease also favoured the intensity of its development.

No substantial differences in the incidence of the disease in different soils were found, but more plants contracted club root in the lighter soils when conditions were less favourable for infection.

The results obtained suggest that while liming in the field may control the disease if the spore load is low, even very heavy applications of lime may not be effective if the soil is heavily contaminated and soil moisture and temperature do not operate as limiting factors.

INTRODUCTION

Field observations have long shown that the incidence and severity of club-root disease caused by *Plasmodiophora brassicae* Woron. are greatly affected by a number of factors. Greenhouse experiments made under controlled conditions to determine the role of such factors have led to more general agreement on the effects of certain of them, but considerable disagreement still exists.

It is now generally accepted that in acid soils favourable conditions for infection and development of the disease are a soil-moisture content of 70–80% of the maximum water-holding capacity (Monteith, 1924; Naumova, 1933) and temperatures falling within the range 18–25° C. (Monteith, 1924; Wellman, 1930).

Naumov (1925) showed that infection depends on the number of spores in the soil and requires a comparatively heavy spore load, but neither he (Naumov, 1928) nor Gibbs (1931) found a direct correlation existing between spore load and the number of infected plants. Disagreeing with this finding, Bremer, Wehnelt & Brandenburg (1937) showed that the number of infected plants varies with spore load up to an upper limit fluctuating with infection conditions. Macfarlane (1952) noted that the percentage of clubbed plants increased rapidly with rising spore concentration, and as higher proportions of plants became infected the rate declined. His results showed that the relationship between severely clubbed plants and spore load is even less marked at high spore loads when an early supply of nutrients at a high level is available. Naumov (1928) and Fedotova (1928) pointed out that in different soils the same spore load gave rise to very different numbers of infected plants.

Early observers associated the disease with a deficiency of lime, but it has been suggested that the inhibiting effect of lime is at least partly associated with some factor other than alkalinity. Naumov (1927), however, showed that the action of lime is not exercised through an effect on host metabolism. Indeed, there is no agreement on the pH range of soils permitting infection, but records exist of infection having occurred within the range pH 4.1–8.8 (Blunck, 1929; Vilkaitis, 1933).

Many workers carrying out greenhouse tests with alkaline soils have used a high moisture content but often without considering other factors such as spore load and temperature. It is therefore difficult to correlate results obtained by different workers, but the literature shows that it is more difficult to obtain heavy attacks in alkaline soil in pots than it is in the field. Lindfors (1924) recorded that in pot tests only a few plants were diseased when the pH was 7.1–7.8 and none if the initial reaction was above pH 7.8. Chupp (1928) found that the upper limit for infection in pots was pH 7.2–7.4. Naumova (1933) recorded infection within a pH range of 5.7–8.4 with an optimum near neutrality, the reduction in infection being most pronounced above 7.0. Larson & Walker (1934) found infection readily occurring in the field at pH 7.1, but in such soil in pot experiments only when its moisture

content fluctuated at relatively low levels or the soil was forcibly aerated. Samuel & Garrett (1945) obtained infection of root hairs in a mixture of sand and soil at pH 7.7 at 25° C. but only with a high spore concentration. Haenseler (1937) suggested that uneven distribution of soil acidity may be adequate to account for the occurrence of field infection in soil giving, with composite samples, a pH of 7.5-7.8 but including small local areas of pH 5.7-8.4.

It is not clear if infection is influenced by the amount of organic matter in the soil, but it has occurred readily in sand cultures (Pryor, 1940). The effect on infection of the supply of various elements essential to the nutrition of the host has been studied by Brejneff (1934), Pryor (1940), Gries, Horsfall & Jacobson (1944) and Walker & Hooker (1945), fairly general agreement resulting.

Biological techniques have already been evolved for evaluating fungicides for the control of certain seed-borne diseases of oats and flax (Muskett, 1938; Muskett & Colhoun, 1942). An investigation was started in 1935 to develop such a technique for evaluating fungicides for the control of *P. brassicae*, but preliminary field experiments demonstrated the difficulty of securing a consistently high level of infection with *P. brassicae* even in acid soils, and therefore tests were planned under controlled conditions. When the work commenced the exact conditions for producing a heavy attack consistently in alkaline soils in pots were not known. Since some fungicides render the soil more alkaline any effect on infection which may be associated with soil pH must be distinguished from direct fungitoxic action. Experiments were therefore made to determine how an epidemic could be consistently produced in either acid or alkaline soils and how attacks of predetermined severity could be induced. A brief summary of the conclusions reached has already been published (Colhoun, 1952).

EXPERIMENTAL TECHNIQUE

Cabbage seedlings (variety Enfield Market) were raised in boxes of sterilized compost, the seed having been disinfected with an organo-mercurial dressing. When the seedlings bore two or three well-developed foliage leaves, usually at the age of 4 or 5 weeks, they were transplanted into either naturally or artificially contaminated soil in water-tight tins (22 cm. diameter, 11.5 cm. high) as employed by Muskett (1937). The water content, and where necessary the pH value of the soil, was adjusted before the transplanting. The pots were kept cool and shaded for 4 days after planting and then moved into their permanent quarters, either in a heated or unheated greenhouse or on an open verandah with a north aspect, according to the temperature range required. In a single experiment at constant temperature the pots were immersed in a thermostatically controlled water-bath.

Soil. From 1935 to 1948 soil naturally contaminated with *P. brassicae* from the grounds of Stranmillis Training College, Belfast, was employed. Susceptible cruciferous crops had been grown in this garden for many years, and, in spite of various lime applications over a considerable period, severe outbreaks of the

disease occurred yearly. Where the heaviest doses of lime had been employed the soil pH in 1935 was 7.8; elsewhere it was 6.3. From 1949 onwards two soils were employed in each experiment—a heavy loam from the Agricultural Research Institute, Hillsborough, Co. Down, and a lighter soil from the grounds of Stormont Castle, Belfast. These soils will be referred to later as the Stranmillis, Hillsborough and Stormont soils. Neither of the two latter soils was naturally contaminated with *P. brassicae*. Adequate bulks of the Hillsborough and Stormont soils were obtained in 1949 to ensure uniform supplies for subsequent work. A fresh supply of the naturally contaminated Stranmillis soil was obtained for each experiment. Each soil was air-dried as required by spreading it in a thin layer on the floor of a heated greenhouse, after which it was sieved and thoroughly mixed. Naturally contaminated soil was used as soon as possible after being brought from the field, but the bulks of uncontaminated soil were stored in a dry room until required.

Adjustment of soil reaction. In experiments on infection in relation to different soil reactions the pH values of the acid Hillsborough and Stormont soils were adjusted as required with chemically pure calcium hydroxide, calcium oxide, calcium carbonate or other alkali base or salt. The chemicals were thoroughly incorporated in the air-dried soil which was then brought to the required moisture content within 24 hr. The Stranmillis soil was used as brought from the field without adjustment of pH value.

Inoculation. Diseased cabbage roots were collected during the winter from Stranmillis. After washing, the swellings caused by *P. brassicae* were cut off, minced, mixed and stored in glass jars at about -3°C . This material provided a source of spores for inoculation and was always used in as fresh a condition as possible. Diseased roots collected in late summer or autumn also provided inoculum for immediate use. When required for inoculation, stored material was gradually brought to laboratory temperatures, after which it was treated in the same manner as fresh material, being ground up in a mortar with water and filtered through fine muslin. The spore concentration per ml. of the suspension was determined with a haemocytometer and adjusted as required. The spore load is always stated as spores per g. of oven-dried soil so as to provide a standard unit. The volume of spore suspension required to provide the desired level of spore load was diluted with water to supply a standard volume per pot. The levels of spore load employed ranged from 1000 to 25,000,000 spores per g. of oven-dried soil.

No spore suspension was added to the naturally contaminated Stranmillis soil. The Stormont and Hillsborough soils were inoculated in all infection experiments and the soil was thoroughly mixed after the addition of the spore suspension.

Moisture control. The maximum water-holding capacity (M.W.H.C.) of each soil was determined by a modification of the box method (Hall, 1931). The M.W.H.C. of the various lots of Stranmillis soil employed ranged from 36 to 53%. The corresponding values for the Hillsborough and Stormont soils were 55 and 43% respectively. The moisture content of each bulk of soil was also determined at the

commencement of an experiment. All moisture contents were determined by drying the soil for 24 hr. at 100° C., the percentage of water present being then calculated on the basis of this oven-dried soil. The amount of water necessary to raise the moisture content of the soil to the desired level, less the volume of liquid already applied as spore suspension, was mixed with the soil. The moisture content was then redetermined. The soil, having been adjusted to the desired moisture content, was placed in pots filled to a standard weight to facilitate subsequent weighing. When very dry and very wet soils were used in the same experiment it was necessary to employ two or more standard pot weights.

After transplanting, the pots were weighed at stated intervals and brought up to the original weight by adding water. In certain experiments weighing of the pots was not carried out after planting had been completed, but known quantities of water were added to each, usually at intervals of 5 days.

Determination of soil pH. Determinations of pH were made with a quinhydrone electrode before 1939 and subsequently with a glass electrode. When the soil pH was adjusted with an alkali base or salt, the redetermination of pH was made at least 5 days later. Determinations were usually made immediately before planting and again at the end of the experiment.

Planting. After being removed from the sterilized soil, the roots of the young plants were thoroughly washed with water. The roots were usually placed in the dibble holes without watering, but in a few experiments water was added. Twenty seedlings were transplanted into each pot unless otherwise stated.

Temperature control. When the transplanted plants had become established they were removed to their permanent quarters. Self-recording thermometers registered the temperature of the air around the pots, but this was fairly close to the soil temperature (see Fig. 1). The air temperatures recorded every 2 hr. were averaged to give the mean for each experiment. Daily temperature averages are given in Fig. 2.

Examination of the plants. The plants were carefully uprooted usually after 4 or 5 weeks' growth in the contaminated soil. In all experiments except in 1951, the plants were divided into two categories only: (a) healthy, and (b) showing macroscopically visible symptoms of attack by *P. brassicae*. In 1951 the degree of attack was recorded in four subgroups: (1) very slight, (2) slight, (3) moderate and (4) very severe. Washing of the roots was sometimes necessary to facilitate this classification. The percentage number of diseased plants, and, when applicable, the percentage number in each subgroup, was calculated for each pot on the basis of the total plants surviving at the end of an experiment. Within the short duration of an experiment plants did not die from infection with *P. brassicae*.

When the diseased plants had been classified into subgroups a disease index was obtained for each pot by multiplying the percentage of plants in each subgroup by the subgroup number and then adding the products. Thus if 100% of the surviving plants were placed in subgroup 4 the disease index would be 400.

RESULTS

*Experiments with naturally contaminated soil**(a) Acid soils*

In preliminary experiments with Stranmillis soil of pH 6.3 during 1935 and 1936 plants were grown during the summer in an unheated greenhouse (mean about 18° C.). The moisture content of the soil was maintained at approximately 70% M.W.H.C. by watering on alternate days. In all such experiments practically every plant showed severe clubbing within 5 weeks of transplanting.

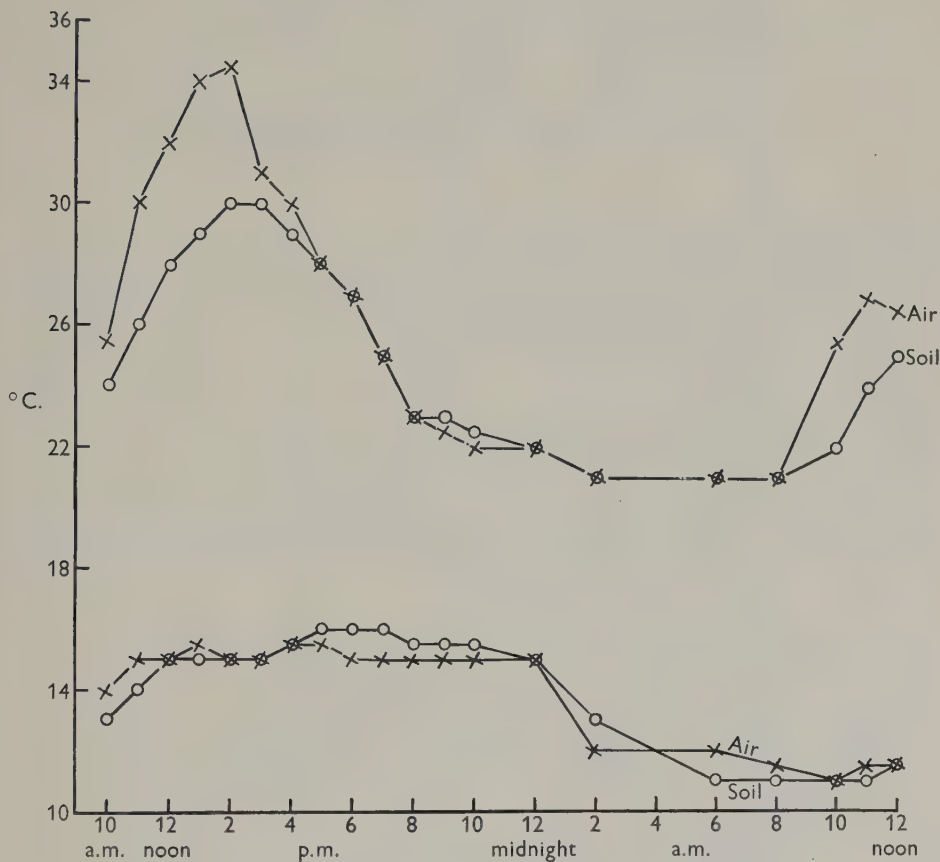


Fig. 1. Relationship between air and soil temperatures in two greenhouse experiments.

Experiment 1. To study the effect of temperature, Stranmillis soil (pH 6.3) at 70% M.W.H.C. was employed, 100 plants being grown in an open-air verandah (mean 14.1° C.; range 5–20° C.) and 100 in the greenhouse (mean 17.8° C.; range 5–31° C.). Fig. 2 shows the mean daily temperatures during the period of the

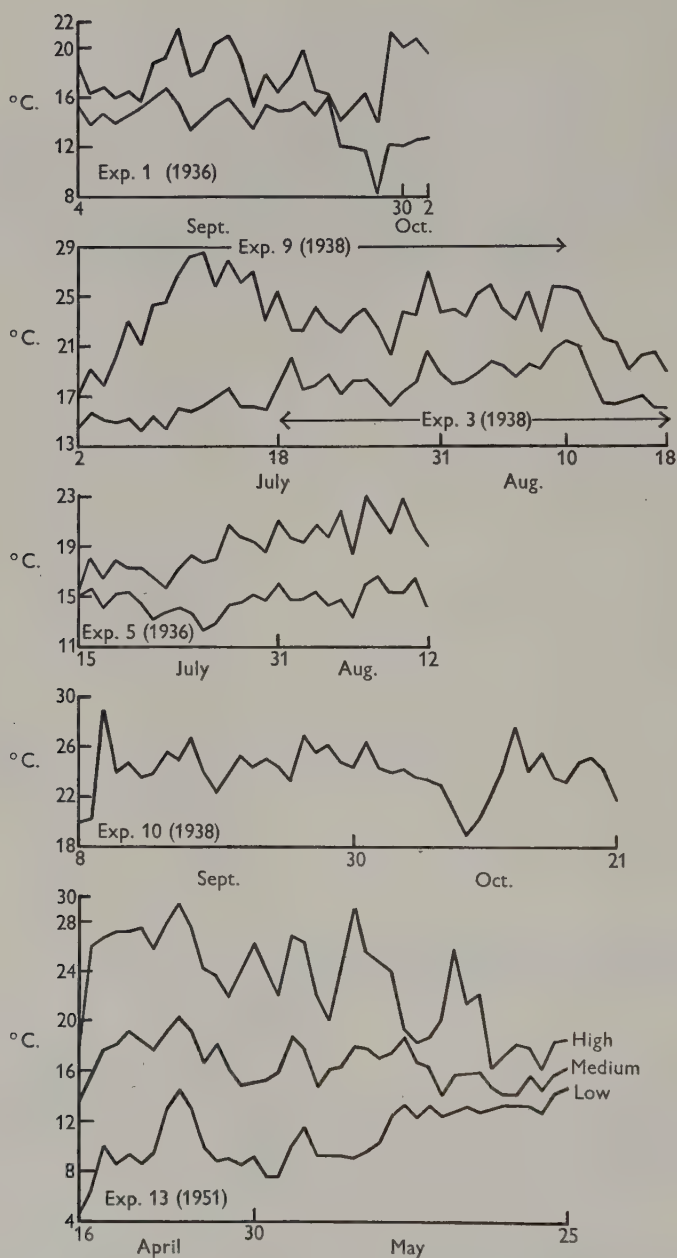


Fig. 2. Mean daily air temperatures for typical experiments.

experiment (4 September–2 October 1936). After 28 days' growth in the contaminated soil all the plants grown in the verandah were healthy, but those in the greenhouse were diseased.

Experiment 2. The effect of moisture content was studied in a heated greenhouse (mean 17.8° C.; range 9–32° C.) with Stranmillis soil of pH 6.3 during the period 6 October–13 November 1936. Pots were prepared with soil at five moisture levels, varying from 40 to 80% M.W.H.C., the pots being watered on alternate days. Table 1 gives the mean percentage number of diseased plants for each soil moisture.

TABLE 1. *Effect of soil moisture, 1936*

Percentage M.W.H.C. of soil	No. of plants surviving (out of 100)	Mean % no. of diseased plants
40	99	6.1
50	95	12.9
60	94	50.6
70	88	80.8
80	90	73.3

Table 1 shows that the percentage number of diseased plants increased with increase of soil moisture between 40 and 70% M.W.H.C., while the largest number of diseased plants occurred at 70%.

Experiment 3. To study the combined effects of temperature and moisture an experiment was made from 18 July to 18 August 1938, using Stranmillis soil of pH 6.3. Soils at 40, 50, 60, 70 and 80% M.W.H.C. were maintained in each of two series of pots. Four pots were set up with soil at each moisture content in each series. One series was in a greenhouse (mean 23.2° C.; range 19–27° C.); the other in a verandah (mean 18.4° C.; range 12–25° C.). Fig. 2 gives the mean daily temperatures for each series; the results obtained are presented in Table 2.

TABLE 2. *Effects of soil moisture and temperature, 1938*

Percentage M.W.H.C. of soil	Greenhouse (23.2° C.)		Verandah (18.4° C.)	
	No. of plants surviving (out of 80)	Mean % no. of diseased plants	No. of plants surviving (out of 80)	Mean % no. of diseased plants
40	79	2.5	77	0
50	79	22.9	73	2.7
60	80	97.5	71	24.3
70	75	97.5	70	56.9
80	77	100.0	73	68.7

Table 2 shows that the most favourable conditions for infection and development of the disease were provided by the damper soils and the higher temperature.

Experiment 4. This experiment on the effect of fluctuating moistures on the incidence of the disease was made at the same time as Exp. 3 with Stranmillis soil

of pH 6.3 and at the same two temperature ranges. Two series of pots, in quadruplicate, were prepared with soil of 30, 40, 50, 60 and 70% M.W.H.C. One series was kept in the greenhouse and the other in the verandah. The moisture content of the dampest soil was maintained at a relatively constant level by watering on alternate days, but that of the remainder was allowed to fluctuate. Each pot with fluctuating soil moisture received 300 ml. of water 5 days after planting and then alternate doses of 600 and 300 ml. at intervals of 5 days in the greenhouse, or 10 days in the verandah. These fluctuations raised the soil moisture of all pots to at least 65% M.W.H.C. during the early part of the experiment. The results obtained are presented in Table 3, which shows that, as in previous experiments, more plants were diseased at the higher temperature. It is also seen that fluctuating moistures are as favourable for the development of the disease as a high constant moisture.

TABLE 3. *Effects of temperature and fluctuating soil moisture, 1938*

Initial % M.W.H.C. of soil	Moisture level	Greenhouse (23.2° C.)		Verandah (18.4° C.)	
		No. of plants surviving (out of 80)	Mean % no. of diseased plants	No. of plants surviving (out of 80)	Mean % no. of diseased plants
30	Fluctuating	75	98.8	75	78.3
40	Fluctuating	78	100.0	75	71.0
50	Fluctuating	74	97.4	72	75.8
60	Fluctuating	80	98.8	73	59.1
70	Constant	80	98.8	71	89.7

(b) *Neutral and alkaline soils*

Preliminary observations made in Stranmillis soil of pH 7.8 at 70% M.W.H.C. showed that a few diseased plants occurred under greenhouse, but none under verandah, growing conditions. The mean temperature calculated for such greenhouse experiments never exceeded 20° C.

Experiment 5. The effect of temperature on the incidence of the disease in Stranmillis soil of pH 7.8 was studied in the following environments: (a) verandah (mean 14.7° C.; range 9–22° C.), (b) greenhouse (mean 19.0° C.; range 9–33° C.) and (c) a thermostatically controlled water-bath (23.0° C.). The soil was maintained at 70% M.W.H.C. by watering on alternate days. Six pots, each containing fifteen plants, constituted a unit under each set of growing conditions. The plants grew in the contaminated soil from 15 July to 12 August 1936, at the mean daily temperatures shown in Fig. 2. The results given in Table 4 show that the number of diseased plants increases with increase in temperature within the limits employed.

Experiment 6. An attempt was made to repeat an experiment carried out by Larson & Walker (1934) who demonstrated that infection occurs readily in soil of pH 7.1 when the soil moisture fluctuates at relatively low levels, but not when a high level of moisture is constantly maintained. In this experiment Stranmillis soil of

pH 7.8 was used, but otherwise the conditions were those specified by Larson & Walker, the correct volume of soil at 16% M.W.H.C. being contained in each box (boxes were employed instead of the usual pots) and 30 ml. of water applied around each seedling at planting. Although the amounts of water stated by Larson & Walker were added to the soil at intervals of 5 days, the plants did not survive in the very dry soil in the verandah and so no results could be obtained.

TABLE 4. *Effects of temperature in alkaline soil, 1936*

Growing conditions	Mean temperature (° C.)	No. of plants surviving (out of 90)	Mean % no. of diseased plants
Verandah	14.7	90	3.3
Greenhouse	19.0	90	24.3
Water-bath	23.0	90	41.1

Experiment 7. To study the effects of fluctuating moisture at various levels this experiment was made with Stranmillis soil of pH 7.7 in the usual pots. Initial soil-moisture contents equal to 16, 25, 30 and 35% M.W.H.C. were employed in each of two series of pots, one series in the greenhouse (mean 19.0° C.; range 9–41° C.) and the other in a verandah (mean 15.0° C.; range 9–24° C.). Plants were watered individually at the time of planting, each of those in a pot receiving 10, 20 or 40 ml. Subsequently fluctuating moisture levels were provided by adding to each pot every 5 days the quantities of water stated in Table 5. Towards the end of the growing period less water was added to pots in the verandah than to corresponding greenhouse pots because the verandah pots lost less water. For purposes of comparison, soil of 70% M.W.H.C. was included in each of the two series, but the moisture content was maintained at a relatively constant level by watering on alternate days, using very dilute solutions of sulphuric acid for certain pots. Four pots constituted a unit for each treatment in each series. The period of growth was from 3 July to 9 August 1937.

Table 5 shows that very few diseased plants occurred in the alkaline soil in either the greenhouse or verandah series when the moisture fluctuated at low levels, but more were recorded, particularly in the greenhouse, when soil moisture fluctuated at higher levels. In the verandah series, soils which were dampest immediately after planting became waterlogged for short periods during the growing period, and this may, in part, account for the small numbers of diseased plants occurring there. A large number of plants was diseased in the acidified soil at a relatively constant high soil moisture under greenhouse conditions, while fewer were diseased in the corresponding pots of untreated soil. Very few diseased plants occurred in the verandah series in alkaline or acidified soil at a constant moisture level.

Experiment 8. The effects of fluctuations in soil moisture at higher levels than those employed in Exp. 7 were investigated using Stranmillis soil of pH 7.8. The experiment was made, in quadruplicate, from 11 September to 11 October 1937 in

TABLE 5. *Effects of fluctuating moisture content of alkaline soil in relation to temperature, 1937*

Initial % M.W.H.C. of soil	Water added at planting (ml. per plant)	Later additions of water at intervals of 5 days (100 ml. per pot)	Growing conditions	No. of plants surviving (out of 80)	Mean % no. of diseased plants
16	10	1, 2, 1, 2.5, 0.5, 2.5, 1, 2.5	Greenhouse	69	0
16	20	1, 2, 1, 2.5, 1, 2.5, 1, 2.5	Greenhouse	74	0
16	40	2, 4, 2, 4, 2, 4, 2, 4	Greenhouse	78	7.6
16	10	1, 2, 1, 2.5, 0.5, 0, 1, 0	Verandah	67	0
16	20	1, 2, 1, 2.5, 1, 0, 1, 0	Verandah	77	1.2
16	40	2, 4, 2, 4, 2, 0, 2, 0	Verandah	65	6.5
25	10	1, 2, 1, 2.5, 1, 2.5, 1, 2.5	Greenhouse	75	1.3
25	20	1, 2, 1, 2.5, 1, 2.5, 1, 2.5	Greenhouse	74	1.5
25	40	2, 4, 2, 4, 2, 4, 2, 4	Greenhouse	76	25.0
25	10	1, 2, 1, 2.5, 1, 0, 1, 0	Verandah	69	0
25	20	1, 2, 1, 2.5, 1, 0, 1, 0	Verandah	69	2.9
25	40	2, 4, 2, 4, 2, 0, 2, 0	Verandah	70	19.6
30	10	0.5, 1, 1, 2.5, 1, 2.5, 1, 2.5	Greenhouse	77	0
30	20	1, 2, 1, 2.5, 2, 2.5, 2, 2.5	Greenhouse	78	6.6
30	40	2, 4, 2, 4, 2, 4, 2, 4	Greenhouse	74	39.1
30	10	0.5, 1, 2, 2.5, 1, 0, 1, 0	Verandah	74	0
30	20	1, 2, 2, 2.5, 2, 0, 2, 0	Verandah	66	2.8
30	40	2, 4, 2, 4, 2, 0, 2, 0	Verandah	68	2.6
35	10	0.5, 1, 2, 2.5, 1, 2.5, 1, 2.5	Greenhouse	76	0
35	10	0.5, 1, 2, 2.5, 1, 0, 1, 0	Verandah	68	0
70	0	Moisture level constant	Greenhouse	77	24.9
70	0	Moisture level constant	Verandah	75	2.8
70	0	Moisture level constant + H ₂ SO ₄	Greenhouse	46	69.8
70	0	Moisture level constant + H ₂ SO ₄	Verandah	50	1.6

Greenhouse: mean temperature 19.0° C.

Verandah: mean temperature 15.0° C.

TABLE 6. *Effects of fluctuating moisture content of alkaline soil, 1937*

Initial % M.W.H.C. of soil	Water added at planting (ml. per plant)	Later additions of water at intervals of 5 days (100 ml. per pot)	No. of plants surviving (out of 80)	Mean % no. of diseased plants
30	20	1.5, 3, 1.5, 3, 1.5	79	2.6
35	20	1.5, 3, 1.5, 3, 1.5	78	0
40	20	1.5, 3, 1.5, 3, 1.5	76	6.6
45	20	1.5, 3, 1.5, 3, 1.5	75	6.7
50	20	1.5, 3, 1.5, 3, 1.5	76	34.1
30	40	2, 4, 2, 4, 2	74	44.1
35	40	2, 4, 2, 4, 2	79	27.3
40	40	2, 4, 2, 4, 2	71	60.3
45	20	2, 4, 2, 4, 2	76	68.5
50	20	2, 4, 2, 4, 2	75	72.3
30	40	3, 5, 3, 5, 3	78	87.1
30	0	3, 6, 3, 6, 3	77	54.4
35	0	3, 6, 3, 6, 3	79	96.1
40	0	3, 6, 3, 6, 3	75	98.1
45	0	3, 6, 3, 6, 3	80	96.3
50	0	3, 6, 3, 6, 3	79	98.7

a heated greenhouse (mean $23.1^{\circ}\text{C}.$; range $11-39^{\circ}\text{C}.$). Table 6 records the moisture conditions provided and lists the results obtained.

In Table 6 the results obtained at rather high temperatures suggest that fluctuations of soil moisture at relatively high levels favour infection more than fluctuations at lower levels.

Experiment 9. The effects of different levels of fluctuating or relatively constant soil moisture in Stranmillis soil of pH 7.7 were investigated. One series of pots was maintained in a greenhouse (mean $23.8^{\circ}\text{C}.$; range $12-41^{\circ}\text{C}.$) and another series in a verandah (mean $17.5^{\circ}\text{C}.$; range $11-25^{\circ}\text{C}.$). The initial moisture contents of the

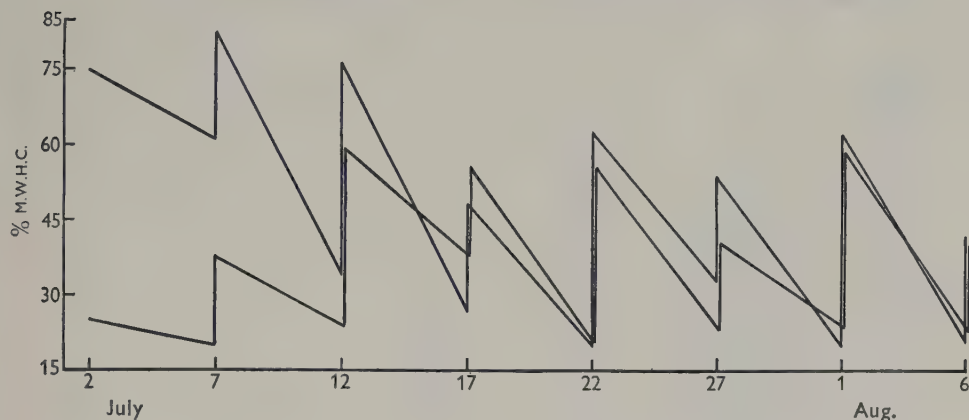


Fig. 3. Soil moisture content in relation to two levels of fluctuating moisture.

soils are stated in Table 7. The relatively constant levels of soil moisture were maintained by watering on alternate days and so bringing the pots to the original weight. The following additions of water (ml. per pot) were made at intervals of 5 days to pots in the greenhouse series to provide soil-moisture fluctuations: 300, 600, 300, 600, 300, 600, 300. In the verandah series the corresponding quantities of water (ml. per pot) added were 300, 0, 600, 0, 0, 300, 0. The water contents of sample cores of soil from pots in which the soil moisture fluctuated were determined immediately before each watering, and from these the moisture contents of the soils after watering were calculated, it being assumed that the soil in a pot was evenly moist immediately before or after watering. Fig. 3 gives the data from the greenhouse series for soils showing the lowest and highest initial moisture contents and shows that after a certain period there was little difference. In the verandah series, the higher initial moisture contents were longer retained. Four pots constituted a unit for each treatment in the greenhouse or verandah. Fig. 2 gives the mean daily temperatures during the growing period (2 July–10 August 1938).

Table 7 shows that in the greenhouse many plants were diseased at all levels of fluctuating soil moisture. A correspondingly large number of diseased plants

occurred at a relatively constant level of moisture only when it was equal to 70% M.W.H.C. Fig. 3 shows that in the greenhouse series the fluctuations in moisture of soil with the lowest initial moisture content provided a level of at least 60% M.W.H.C. at an early stage in the growing period. In the verandah series most diseased plants occurred where the moisture fluctuated at high levels. The results obtained again demonstrate that a severe attack of the disease can occur in alkaline soil when a high temperature and a high level of moisture are provided.

TABLE 7. *Comparison of effects of different levels of relatively constant and fluctuating soil moistures of alkaline soil at two temperature ranges, 1938*

Initial % M.W.H.C. of soil	Moisture level	Greenhouse (23·8° C.)		Verandah (17·5° C.)	
		No. of plants surviving (out of 80)	Mean % no. of diseased plants	No. of plants surviving (out of 80)	Mean % no. of diseased plants
25	Fluctuating	55	90·0	69	5·8
30	Fluctuating	73	70·5	66	7·2
35	Fluctuating	68	86·2	68	6·0
40	Fluctuating	79	59·7	71	8·0
45	Fluctuating	76	72·4	74	1·3
50	Fluctuating	77	51·9	74	6·8
55	Fluctuating	72	68·1	72	7·2
60	Fluctuating	75	68·8	69	9·1
65	Fluctuating	73	61·6	70	22·1
70	Fluctuating	77	86·9	74	32·8
75	Fluctuating	77	90·8	70	37·9
30	Constant	79	0	71	0
40	Constant	78	3·8	67	1·7
50	Constant	75	33·1	69	0
60	Constant	78	36·7	70	2·7
70	Constant	77	92·2	73	12·5

Experiment 10. This experiment compared the effect of fluctuating moisture with more constant levels than used in previous experiments. Stranmillis soil of pH 7·8 was used and the treatments given are stated in Table 8. The experiment was made in a heated greenhouse (mean 24·0° C.; range 19–29° C.) from 8 September to 21 October 1938, the mean daily temperatures being recorded in Fig. 2. Four pots constituted a unit for each treatment. Table 8 shows that fewer diseased plants occurred than in Exp. 9 at similar moisture levels. This may be due to higher mean daily temperatures shortly after transplanting in Exp. 9. The results suggest that a fluctuating high level of soil moisture was not more favourable for the incidence of the disease than a constant moisture level. Few diseased plants occurred when the soil moisture fluctuated at low levels.

Experiment 11. From 28 May to 30 June 1948, an experiment was made using Stranmillis soil of pH 6·8 at six levels of initial moisture content and with three methods of controlling the moisture level: (a) watering four times daily, (b) watering on alternate days and (c) adding known quantities of water, 300 and 600 ml. per pot

TABLE 8. *Comparison of effects of different levels of constant and fluctuating soil moistures of alkaline soil, 1938*

Initial M.W.H.C. of soil	Moisture level	Frequency of weighing and watering	Additions of water (100 ml. per pot) at intervals of 5 days	No of plants surviving (out of 80)	Mean % no. of diseased plants
70	Constant	2 daily	—	76	30.5
70	Constant	1 daily	—	75	34.8
70	Constant	Alternate days	—	76	57.2
70	Constant	Every 3 days	—	73	52.3
70	Constant	Every 4 days	—	71	38.3
70	Constant	Every 5 days	—	72	30.8
40	Constant	2 daily	—	70	0
50	Constant	2 daily	—	66	0
60	Constant	2 daily	—	59	14.8
70	Constant	2 daily	—	78	19.2
40	Fluctuating	—	3, 6, 3, 6, 3, 6, 3, 6	72	4.1
50	Fluctuating	—	3, 6, 3, 6, 3, 6, 3, 6	64	19.1
60	Fluctuating	—	3, 6, 3, 6, 3, 6, 3, 6	65	27.9
70	Fluctuating	—	3, 6, 3, 6, 3, 6, 3, 6	76	24.1
70	Fluctuating	—	4, 8, 4, 8, 4, 8, 4, 8	66	13.3*
50	Fluctuating	—	1, 2, 1, 2, 1, 2, 1, 2	73	0
50	Fluctuating	—	1.5, 3, 1.5, 3, 1.5, 3, 1.5, 3	73	0
50	Fluctuating	—	2.5, 5, 2.5, 5, 2.5, 5, 2.5, 5	69	0
50	Fluctuating	—	4, 8, 4, 8, 4, 8, 4, 8	70	26.8

* Soil became waterlogged at times and plants made poor growth.

TABLE 9. *Comparison of effects of different levels of constant and fluctuating soil moistures of alkaline soil, 1948*

Initial % M.W.H.C. of soil	Moisture level	Method of maintaining soil moisture level	Greenhouse (22.5° C.)		Verandah (12.8° C.)	
			No. of plants surviving (out of 80)	Mean % no. of diseased plants	No. of plants surviving (out of 80)	Mean % no. of diseased plants
27	Constant	4 waterings daily	68	0	75	0
34	Constant	4 waterings daily	80	0	77	0
46	Constant	4 waterings daily	77	7.9	77	1.4
53	Constant	4 waterings daily	78	37.0	75	0
60	Constant	4 waterings daily	78	65.3	75	2.5
70	Constant	4 waterings daily	70	85.3	72	8.5
27	Constant	Watering on alternate days	66	0	68	0
34	Constant	Watering on alternate days	79	5.0	77	0
46	Constant	Watering on alternate days	78	20.6	78	0
53	Constant	Watering on alternate days	80	56.2	79	0
60	Constant	Watering on alternate days	77	77.9	74	2.9
70	Constant	Watering on alternate days	76	97.4	74	0
27	Fluctuating	Doses at intervals of 5 days	26	73.9	53	8.2
34	Fluctuating	Doses at intervals of 5 days	69	91.2	78	10.5
46	Fluctuating	Doses at intervals of 5 days	78	97.4	75	6.9
53	Fluctuating	Doses at intervals of 5 days	77	97.4	72	8.4
60	Fluctuating	Doses at intervals of 5 days	78	96.1	72	0
70	Fluctuating	Doses at intervals of 5 days	76	78.9	72	2.9

alternating at intervals of 5 days. Similar series of pots were maintained in a greenhouse (mean 22.5°C. ; range $8\text{--}39^{\circ}\text{C.}$) and in a verandah (mean 12.8°C. ; range $3\text{--}22^{\circ}\text{C.}$). Four pots constituted a unit for each treatment. The results obtained are stated in Table 9.

As before, the data in Table 9 demonstrate that severe attacks by *P. brassicae* in limed soils occur best at high temperatures, and that at high temperatures infection is favoured equally by a high constant soil moisture or a moisture content fluctuating at high levels.

Experiments with artificially contaminated soil

The investigation was discontinued between 1939 and 1947 and, when work was recommenced in 1948, it was found impossible to obtain consistent results from alkaline Stranmillis soil, in which susceptible cruciferous crops had not been grown for a number of years. It was therefore decided to use soil from Hillsborough and Stormont artificially contaminated with known spore loads.

Preliminary experiments demonstrated that in alkaline soils carrying heavy spore loads severe attacks occurred when the soil pH was adjusted to about 7.5 by the use of calcium hydroxide, calcium oxide, calcium carbonate, sodium carbonate or potassium carbonate.

Experiment 12. To compare the effects of constant and fluctuating soil moistures in relation to spore load and the use of various forms of lime this experiment was made from 10 July to 11 August 1950. The initial moisture content of the soil in all pots corresponded to 70% M.W.H.C. and, as stated in Table 10, four methods of controlling the moisture level were employed. Four levels of spore load were used to provide 10^3 , 10^5 , 10^7 and 2.5×10^7 spores per g. of oven-dried soil. The two acid soils were adjusted to about pH 7.5 with calcium hydroxide, oxide or carbonate; treated and untreated soils were included. Since 128 pots were required, one pot constituted the unit for each treatment. The pots were all maintained in a heated greenhouse (mean 21.7°C. ; range $12\text{--}42^{\circ}\text{C.}$). Table 10 gives the results obtained, together with the pH values of soils at the beginning and end of the experiment. This table shows that the effect of spore load on the number of diseased plants was much greater in alkaline than in acid soils. Very few plants were diseased in alkaline soils containing the two lower spore loads, but more when the spore load was increased. Fewer diseased plants were recorded when calcium carbonate was used instead of calcium hydroxide or oxide, probably because the pH value of the soil decreased less during the growing period when calcium carbonate was employed. The results did not suggest any consistent differences between the effects of constant and of fluctuating levels of soil moisture.

Experiment 13. The interaction of the effects of temperature, moisture, spore load and soil reaction was studied in this experiment made in three greenhouses from 16 April to 25 May 1951. Three temperature ranges were provided as follows: (a) high (mean 23.0°C. ; range $10\text{--}40^{\circ}\text{C.}$), (b) medium (mean 16.6°C. ; range $9\text{--}33^{\circ}\text{C.}$) and (c) low (mean 10.8°C. ; range $1\text{--}19^{\circ}\text{C.}$). Four levels of spore

TABLE 10. *Effects in alkaline and acid soils (artificially contaminated) of different levels of spore load at constant and fluctuating moisture contents, 1950*

Percentage no. of diseased plants per pot									
Soil	Amount of lime added per pot*	Soil pH		Spore load (per g. of oven-dried soil)	Constant moisture (pots weighed and watered 4 times daily)	Relatively constant moisture (pots weighed and watered on alternate days)	Fluctuating moisture (pots weighed and watered at intervals of 5 days)	Fluctuating moisture (additions of 300 ml. water per pot alternating at intervals of 5 days)	
		At planting	At end of exp.						
Stormont	Untreated	5.3-5.4	5.1-5.6	10 ³	94.1	77.7	100.0	68.4	
	Untreated	5.3-5.4	5.1-5.6	10 ⁵	66.3	100.0	100.0	100.0	
	Untreated	5.3-5.4	5.1-5.6	10 ⁷	100.0	100.0	100.0	85.0	
	Untreated	5.3-5.4	5.1-5.6	2.5 × 10 ⁷	93.7	100.0	100.0	95.0	
Hillsborough	Untreated	5.1-5.2	4.7-5.3	10 ³	73.7	27.7	31.2	0	
	Untreated	5.1-5.2	4.7-5.3	10 ⁵	94.4	100.0	89.4	85.0	
	Untreated	5.1-5.2	4.7-5.3	10 ⁷	95.0	100.0	94.1	89.4	
	Untreated	5.1-5.2	4.7-5.3	2.5 × 10 ⁷	94.7	90.0	94.1	64.7	
Stormont	Ca(OH) ₂ , 16.0 g.	7.4-7.5	6.8-7.2	10 ³	0	0	0	5.0	
	Ca(OH) ₂ , 16.0 g.	7.4-7.5	6.8-7.2	10 ⁵	10.0	7.7	60.0	10.5	
	Ca(OH) ₂ , 16.0 g.	7.4-7.5	6.8-7.2	10 ⁷	60.0	88.8	95.0	47.4	
	Ca(OH) ₂ , 16.0 g.	7.4-7.5	6.8-7.2	2.5 × 10 ⁷	90.0	88.2	88.2	72.2	
Hillsborough	Ca(OH) ₂ , 16.0 g.	7.4-7.5	6.4-6.9	10 ³	0	0	0	11.1	
	Ca(OH) ₂ , 16.0 g.	7.4-7.5	6.4-6.9	10 ⁵	16.6	16.6	5.9	0	
	Ca(OH) ₂ , 16.0 g.	7.4-7.5	6.4-6.9	10 ⁷	94.1	72.2	73.7	45.0	
	Ca(OH) ₂ , 16.0 g.	7.4-7.5	6.4-6.9	2.5 × 10 ⁷	66.7	73.7	40.0	63.2	
Stormont	CaO, 13.5 g.	7.3-7.5	6.9-7.1	10 ³	0	0	0	5.0	
	CaO, 13.5 g.	7.3-7.5	6.9-7.1	10 ⁵	12.5	11.1	10.0	15.0	
	CaO, 13.5 g.	7.3-7.5	6.9-7.1	10 ⁷	72.2	58.8	78.9	90.0	
	CaO, 13.5 g.	7.3-7.5	6.9-7.1	2.5 × 10 ⁷	90.0	90.0	77.7	73.7	
Hillsborough	CaO, 13.5 g.	7.4-7.6	6.5-6.7	10 ³	6.7	5.6	5.9	0	
	CaO, 13.5 g.	7.4-7.6	6.5-6.7	10 ⁵	11.1	15.0	5.5	11.8	
	CaO, 13.5 g.	7.4-7.6	6.5-6.7	10 ⁷	57.1	94.1	76.4	66.6	
	CaO, 13.5 g.	7.4-7.6	6.5-6.7	2.5 × 10 ⁷	82.3	68.4	82.3	42.1	
Stormont	CaCO ₃ , 33.0 g.	7.3-7.5	7.6-7.8	10 ³	0	0	0	0	
	CaCO ₃ , 33.0 g.	7.3-7.5	7.6-7.8	10 ⁵	0	10.5	0	0	
	CaCO ₃ , 33.0 g.	7.3-7.5	7.6-7.8	10 ⁷	5.9	10.5	0	26.3	
	CaCO ₃ , 33.0 g.	7.3-7.5	7.6-7.8	2.5 × 10 ⁷	17.6	68.4	5.9	30.0	
Hillsborough	CaCO ₃ , 33.0 g.	7.4-7.5	7.1-7.3	10 ³	0	0	0	0	
	CaCO ₃ , 33.0 g.	7.4-7.5	7.1-7.3	10 ⁵	12.5	5.0	0	5.9	
	CaCO ₃ , 33.0 g.	7.4-7.5	7.1-7.3	10 ⁷	27.8	22.2	5.3	0	
	CaCO ₃ , 33.0 g.	7.4-7.5	7.1-7.3	2.5 × 10 ⁷	26.3	31.6	33.3	0	

* In this, and in subsequent experiments, each pot contained the equivalent of 3000 g. oven-dried soil.

load varying from 10^3 to 2.5×10^7 spores per g. of oven-dried soil and moisture contents corresponding to 40, 50, 60 and 70% M.W.H.C. of the soils were employed. The moisture contents were maintained by watering on alternate days. Acid soils were included as well as those treated with alkali to adjust the pH value to approximately 7.5. One pot constituted a unit for each treatment and 192 pots were used. Fig. 2 gives the mean daily temperatures, and Table 11 assembles the percentage number of diseased plants in each pot, together with data for disease index and soil pH.

From the results stated in Table 11 the following points are noted:

(1) When suitable conditions were provided, infection and development of the disease occurred in soils showing a pH of about 7.5 at the time of planting and not below 7.0 at the conclusion of the experiment.

(2) At low temperatures plants did not become diseased irrespective of soil reaction or spore load.

(3) Both medium and high temperatures afforded ideal conditions for infection in acid soils, but in alkaline soils the higher temperature was more favourable.

(4) In acid soils at high temperatures the disease was most severe under high moisture conditions; this relationship was even more pronounced in alkaline soils.

(5) In acid soils of high moisture content at high temperatures the incidence of the disease was little, if at all, affected by the spore load.

(6) In dry acid soils severe attacks of the disease at high temperatures were usually associated with high spore loads.

(7) In wet, acid Stormont soil, at medium temperatures, the number of diseased plants was unaffected by the spore load, but in acid Hillsborough soil, under similar conditions, the severity of the disease increased with increasing spore load.

(8) In alkaline soils the most severe attacks were usually associated with the higher spore loads at all temperature and moisture ranges employed.

(9) Where conditions were less favourable for infection fewer plants, in general, were diseased in the Hillsborough than in the Stormont soil.

(10) Plants grown under those conditions most favourable for infection showed the highest disease index (see p. 266), suggesting that these conditions also favoured the development of frequent and large clubs on the roots.

Experiment 14. The previous experiment was repeated between 6 November 1950 and 5 January 1951 and similar data were collected. Under these unfavourable winter conditions the disease was not so severe as in summer. Nevertheless, the results obtained in the two experiments showed considerable agreement except that in the winter experiment severe attacks occurred in acid soils, even at high temperatures, only in the presence of high spore loads.

Experiment 15. To determine whether diseased plants could occur at soil pH values above 7.5 an experiment was made between 16 April and 25 May 1951. The soils were treated with the amounts of calcium hydroxide or carbonate stated in Table 12 in which the effects of such treatments are shown. This experiment was made at the high temperature range at the same time as Exp. 13 (see Fig. 2). The

soil was maintained at a moisture content equal to 70% M.W.H.C. by weighing on alternate days. The spore load employed was 2.5×10^7 spores per g. of soil. One pot constituted a unit for each treatment.

Table 12 shows that the highest pH value of soil in which the disease appeared was 7.8 (pH 7.1 at the end of the experiment) in the Stormont soil, and pH 7.9 (pH 7.2 at the end of the experiment) in Hillsborough soil. In both, the soil reaction had been adjusted by the addition of calcium hydroxide. The disease index values indicate that with increasing calcium hydroxide or carbonate in the soil the size and number of the swellings on the plant roots were reduced.

TABLE 11. *Interaction of effects of temperature, soil moisture, spore load and soil reaction on incidence of diseased plants, 1951*

A. *At three temperature ranges (high, medium and low) and with a soil moisture equal to 70 % of the M.W.H.C.*

Amount of lime added per pot	Spore load (per g. oven-dried soil)	Soil pH		% no. diseased plants Temperature range			Disease index Temperature range		
		At planting	At end of exp.	High (23.0° C.)	Medium (16.6° C.)	Low (10.8° C.)	High (23.0° C.)	Medium (16.6° C.)	Low (10.8° C.)
Stormont soil									
Untreated	10 ³	4.8-5.0	4.7-4.8	100.0	100.0	0	360	340	0
Untreated	10 ⁵	4.8-5.0	4.7-4.8	100.0	100.0	0	300	360	0
Untreated	10 ⁷	4.8-5.0	4.6-4.9	100.0	100.0	0	365	300	0
Untreated	2.5 × 10 ⁷	4.8-5.0	4.8-5.1	100.0	100.0	0	370	396	0
Ca(OH) ₂ , 16.0 g.	10 ³	7.5-7.6	6.8-7.0	60.0	13.3	0	165	20	0
Ca(OH) ₂ , 16.0 g.	10 ⁵	7.5-7.6	6.9-7.0	95.0	5.0	0	250	5	0
Ca(OH) ₂ , 16.0 g.	10 ⁷	7.5-7.6	6.8-7.0	100.0	50.0	0	290	130	0
Ca(OH) ₂ , 16.0 g.	2.5 × 10 ⁷	7.5-7.6	6.8	100.0	75.0	0	330	215	0
CaO, 13.5 g.	10 ³	7.6-7.8	6.9-7.0	15.0	0	0	30	0	0
CaO, 13.5 g.	10 ⁵	7.6-7.8	6.9-7.0	45.0	0	0	125	0	0
CaO, 13.5 g.	10 ⁷	7.6-7.8	6.8-7.0	100.0	47.4	0	350	153	0
CaO, 13.5 g.	2.5 × 10 ⁷	7.6-7.8	6.7-7.1	100.0	60.0	0	360	140	0
CaCO ₃ , 33.0 g.	10 ³	7.5-7.6	7.4-7.6	5.0	0	0	5	0	0
CaCO ₃ , 33.0 g.	10 ⁵	7.5-7.6	7.4	10.0	0	0	10	0	0
CaCO ₃ , 33.0 g.	10 ⁷	7.5-7.6	7.4	65.0	10.0	0	155	20	0
CaCO ₃ , 33.0 g.	2.5 × 10 ⁷	7.5-7.6	7.3-7.4	80.0	10.5	0	215	32	0
Hillsborough soil									
Untreated	10 ³	5.1-5.2	4.3-5.0	84.2	25.0	0	253	25	0
Untreated	10 ⁵	5.1-5.2	4.4-4.7	95.0	72.2	0	335	250	0
Untreated	10 ⁷	5.1-5.2	4.4-4.5	89.5	83.3	0	347	234	0
Untreated	2.5 × 10 ⁷	5.1-5.2	4.3-4.8	100.0	100.0	0	380	330	0
Ca(OH) ₂ , 16.0 g.	10 ³	7.5-7.6	6.2-6.5	30.0	0	0	50	0	0
Ca(OH) ₂ , 16.0 g.	10 ⁵	7.5-7.6	6.2-6.5	75.0	5.0	0	195	20	0
Ca(OH) ₂ , 16.0 g.	10 ⁷	7.5-7.6	6.2-6.4	80.0	10.0	0	135	20	0
Ca(OH) ₂ , 16.0 g.	2.5 × 10 ⁷	7.5-7.6	6.3-6.4	85.0	5.0	0	190	5	0
CaO, 13.5 g.	10 ³	7.6-7.7	6.2-6.4	25.0	0	0	25	0	0
CaO, 13.5 g.	10 ⁵	7.6-7.7	6.2-6.5	50.0	0	0	95	0	0
CaO, 13.5 g.	10 ⁷	7.6-7.7	6.2-6.5	70.0	15.0	0	180	30	0
CaO, 13.5 g.	2.5 × 10 ⁷	7.6-7.7	6.2-6.6	60.0	10.0	0	80	50	0
CaCO ₃ , 33.0 g.	10 ³	7.4-7.6	7.1-7.2	0	0	0	0	0	0
CaCO ₃ , 33.0 g.	10 ⁵	7.4-7.6	7.0-7.1	15.0	0	0	15	0	0
CaCO ₃ , 33.0 g.	10 ⁷	7.4-7.6	7.0-7.1	15.0	10.5	0	30	10	0
CaCO ₃ , 33.0 g.	2.5 × 10 ⁷	7.4-7.6	7.0-7.1	36.8	15.0	0	99	20	0

TABLE II (cont.)

B. At a high temperature (23.0° C.) range only and with soil moistures equal to 40, 50 and 60% of the M.W.H.C.

Amount of lime added per pot	Spore load (per g. oven-dried soil)	Soil pH		% no. diseased plants % M.W.H.C. of soil			Disease index % M.W.H.C. of soil		
		At planting	At end of exp.						
				40	50	60	40	50	60
Stormont soil									
Untreated	10 ³	4.8-5.0	5.0-5.2	35.0	5.0	100.0	45	5	285
Untreated	10 ⁵	4.8-5.0	5.0-5.3	15.8	30.0	100.0	26	85	320
Untreated	10 ⁷	4.8-5.0	5.0-5.3	75.0	70.0	85.0	200	145	235
Untreated	2.5 × 10 ⁷	4.8-5.0	5.0-5.2	63.2	75.0	73.7	158	190	205
Ca(OH) ₂ , 16.0 g.	10 ³	7.5-7.6	6.8-6.9	0	0	10.0	0	0	15
Ca(OH) ₂ , 16.0 g.	10 ⁵	7.5-7.6	6.9-7.0	15.8	47.1	80.0	26	84	190
Ca(OH) ₂ , 16.0 g.	10 ⁷	7.5-7.6	6.7-6.9	20.0	40.0	95.0	30	65	300
Ca(OH) ₂ , 16.0 g.	2.5 × 10 ⁷	7.5-7.6	6.7-6.9	0	40.0	75.0	0	70	200
CaO, 13.5 g.	10 ³	7.6-7.8	6.9-7.0	0	0	25.0	0	0	60
CaO, 13.5 g.	10 ⁵	7.6-7.8	6.9-7.1	0	15.0	20.0	0	35	40
CaO, 13.5 g.	10 ⁷	7.6-7.8	6.9-7.0	30.0	61.1	100.0	55	147	205
CaO, 13.5 g.	2.5 × 10 ⁷	7.6-7.8	6.9-7.0	5.0	50.0	80.0	10	130	155
CaCO ₃ , 33.0 g.	10 ³	7.5-7.6	7.2	0	0	0	0	0	0
CaCO ₃ , 33.0 g.	10 ⁵	7.5-7.6	7.3	0	0	0	0	0	0
CaCO ₃ , 33.0 g.	10 ⁷	7.5-7.6	7.0-7.3	0	25.0	10.0	0	45	30
CaCO ₃ , 33.0 g.	2.5 × 10 ⁷	7.5-7.6	7.1-7.3	0	0	45.0	0	0	45
Hillsborough soil									
Untreated	10 ³	5.1-5.2	4.9-5.1	0	40.0	31.6	0	70	53
Untreated	10 ⁵	5.1-5.2	5.0-5.2	47.1	15.0	50.0	141	40	160
Untreated	10 ⁷	5.1-5.2	4.9-5.1	15.0	68.4	70.0	15	153	205
Untreated	2.5 × 10 ⁷	5.1-5.2	5.0-5.2	15.0	60.0	85.0	30	90	170
Ca(OH) ₂ , 16.0 g.	10 ³	7.5-7.6	6.3-6.5	0	0	5.0	0	0	10
Ca(OH) ₂ , 16.0 g.	10 ⁵	7.5-7.6	6.1-6.5	0	5.0	25.0	0	5	50
Ca(OH) ₂ , 16.0 g.	10 ⁷	7.5-7.6	6.0-6.7	0	45.0	80.0	10	70	125
Ca(OH) ₂ , 16.0 g.	2.5 × 10 ⁷	7.5-7.6	6.4-6.8	0	5.0	55.0	0	5	90
CaO, 13.5 g.	10 ³	7.6-7.7	6.5-6.6	0	0	10.0	0	0	10
CaO, 13.5 g.	10 ⁵	7.6-7.7	6.3-6.8	0	5.0	25.0	0	5	40
CaO, 13.5 g.	10 ⁷	7.6-7.7	6.4-6.8	0	15.0	30.0	0	25	35
CaO, 13.5 g.	2.5 × 10 ⁷	7.6-7.7	6.4-6.8	10.0	30.0	35.0	10	55	70
CaCO ₃ , 33.0 g.	10 ³	7.4-7.6	7.0-7.4	0	0	0	0	0	0
CaCO ₃ , 33.0 g.	10 ⁵	7.4-7.6	7.0-7.3	0	0	0	0	0	0
CaCO ₃ , 33.0 g.	10 ⁷	7.4-7.6	7.1-7.2	5.0	5.0	10.0	5	5	10
CaCO ₃ , 33.0 g.	2.5 × 10 ⁷	7.4-7.6	7.0-7.4	5.0	5.0	5.0	5	5	5

DISCUSSION

The results obtained in this study have confirmed those obtained by Monteith (1924), Wellman (1930) and Naoumova (1933) for the effects of temperature and moisture on infection in acid soils. It is of interest that fluctuating moisture levels providing wet conditions for short periods favour infection equally as well as constant high soil moistures, for Wellman (1930) demonstrated that the disease resulted when cabbage roots were exposed for at least 18 hr. to wet contaminated soil. The results also indicate the importance of the interaction of factors influencing infection in acid soils and may assist in explaining why earlier workers obtained apparently anomalous results when studying the effect of spore load as a single factor.

TABLE 12. *Effect of high pH values of soil on infection and development of the disease, 1951*

Soil	Amount of lime added per pot	Soil pH		No. of plants surviving (out of 20)	% no. of diseased plants	Disease index (maximum 400)
		At planting	At end of exp.			
Stormont	Ca(OH) ₂ : 18 g.	7.7	6.8	20	80.0	235
	20 g.	7.8	6.9	20	25.0	25
	22 g.	7.8	7.1	20	45.0	45
	24 g.	8.0	7.2	20	0	0
	26 g.	8.1	7.3	20	0	0
	CaCO ₃ : 37 g.	7.3	7.3	19	36.8	84
	41 g.	7.3	7.6	19	15.8*	37*
	45 g.	7.3	7.4	20	35.0	48
	49 g.	7.4	7.4	20	10.0	10
	53 g.	7.3	7.2	19	0	0
Hillsborough	Ca(OH) ₂ : 18 g.	7.7	6.7	20	75.0	180
	20 g.	7.7	6.7	20	55.0	85
	22 g.	7.8	6.8	20	20.0	20
	24 g.	7.9	7.2	20	15.0	15
	26 g.	7.9	7.3	20	0	0
	CaCO ₃ : 37 g.	7.3	7.2	20	50.0	95
	41 g.	7.2	7.1	20	70.0	85
	45 g.	7.3	7.3	19	10.5	10
	49 g.	7.3	7.2	20	20.0	40
	53 g.	7.3	7.3	16	31.3	44

* Low value may be due to a leak in the tin rendering it difficult to maintain the proper moisture level.

Confirmation of results secured by Larson & Walker (1934) in their study of the effect of soil moisture on infection in alkaline soils has not been obtained. They reported infection occurring in the field at pH 7.1, but in greenhouse experiments it did not result at high, intermediate or low constant moisture levels although it occurred when the soil moisture fluctuated at relatively low levels. The present work shows clearly that at high constant or fluctuating moistures infection occurred at pH 7.8 in pot experiments with naturally contaminated soil following liming of the field for many years, or with artificially contaminated soil where the pH was adjusted shortly before planting. On the other hand, low fluctuating or constant soil moisture checked or prevented infection. From the results now presented, it can be concluded that the conditions favouring the occurrence of severe attacks in alkaline soils are more strictly circumscribed than in acid soils, and support is also forthcoming for the contention of Naoumova (1933) that the intensity of infection is a function of many intimately connected external factors.

Temperature has been found in this study to have a greater effect on infection in alkaline than in acid soils. Since very severe attacks occurred in alkaline soils at a *mean* air temperature of 23° C., the temperature range provided was obviously very suitable for infection. During such experiments the temperature frequently exceeded 23° C. by a considerable margin, but in a single experiment when

a constant temperature of 23° C. was maintained only 41% of the plants were infected at pH 7.8. It may therefore be concluded that the optimum temperature for infection in alkaline soils is higher than 23° C., although no results at constant temperatures above this point are available. It is not yet possible to indicate the length of time required at a suitable temperature for infection to occur. Samuel & Garrett (1945) obtained infection of root-hairs at pH 7.7 in a mixture of soil and sand at 25° C., but made no observations on the incidence of clubbing and did not employ other temperatures. In considering the relationship between clubbing and root-hair infection it may be borne in mind that Macfarlane (1952) suggested that clubbing may be influenced by soil conditions subsequent to the first root-hair infections such as those recorded by Samuel & Garrett.

Spore load has now been shown to be a very important factor influencing infection in alkaline soils, heavy attacks being associated only with high spore loads. The relationship between the number of diseased plants in alkaline soils and spore load is therefore of a similar nature to that existing in acid soils where some other factor is unfavourable for infection or good growth of the host.

When all the factors affecting the incidence of club-root are not controlled it is difficult, if not impossible, to obtain consistent results for the upper pH limit or for the maximum lime content of soils in which the disease occurs. It is hoped that the results now presented may assist in explaining why severe attacks have been recorded in the field in limed soils of pH values higher than those regarded by many workers as inhibiting infection in pot experiments. It may also now be suggested that the disease can be controlled under field conditions by liming if the spore load is low, but where the spore load is high even heavy liming may not prevent infection if moisture and temperature are sufficiently high for them not to operate as limiting factors. It is, of course, realized that if biologic races of the parasite exist there is the possibility that such races may vary in their powers of infection at different pH levels.

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SAPROPHYTIC BEHAVIOUR OF SOME CEREAL ROOT-ROT FUNGI

I. SAPROPHYTIC COLONIZATION OF WHEAT STRAW

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The ability of four cereal root-rot fungi, *Helminthosporium sativum*, *Curvularia ramosa*, *Ophiobolus graminis* and *Fusarium culmorum*, to colonize wheat straw as saprophytes has been investigated. Test pieces of straw were buried in a graded dilution series of maize meal-sand culture of the test fungus with unsterilized soil. For assessing percentage straws colonized, three methods were compared: (1) isolation on agar plates, (2) Garrett's (1938*b*) wheat-seedling test and (3) incubation on moist sand, to promote sporulation (the 'sand-plate' test). *Curvularia ramosa* and *Fusarium culmorum* were found to behave as vigorous competitive saprophytes of the soil-inhabiting type, whereas *Helminthosporium sativum* and *Ophiobolus graminis* proved to be weak saprophytic colonizers of the specialized root-inhabiting type.

INTRODUCTION

Since the original conception of a soil microflora, comprising regular 'soil inhabitants' and more specialized 'soil invaders', was advanced by Waksman (1917), attempts have been made to classify soil-borne fungi on an ecological basis (Reinking & Manns, 1933; Burges, 1939; Garrett, 1938*a*, 1944). Recently, Garrett (1950) has reviewed the interrelationships between root-disease fungi and the associated soil microflora and has amended previous classifications to distinguish between specialized root-inhabiting parasites and unspecialized soil-inhabiting parasites. The former are considered to be predominantly host-borne with a limited capacity for saprophytic colonization in the soil-borne phase. Unspecialized parasites, on the other hand, though restricted in their parasitic activities, are vigorous colonizers of dead plant tissues in the soil.

Our present knowledge of the saprophytic behaviour of the majority of root-disease fungi is limited, and comparatively few can be assigned without question to either of the above groups. As Garrett (1950) has already pointed out, such classification is necessary before the relationships between root-infecting fungi and other soil micro-organisms can be elucidated and adequate disease control measures devised.

Mitchell, Hooton & Clark (1941) demonstrated that *Phymatotrichum omnivorum* was unable to colonize sterilized cotton roots inoculated simultaneously with a 'pinch' of unsterilized soil, although it could colonize sterilized roots in pure culture. On the other hand, both Sadasivan (1939) and Walker (1941) isolated

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Fusarium culmorum from fresh wheat straw buried in different types of soil, thus demonstrating that this fungus behaved as a soil-inhabiting saprophyte. The work of Subramanian (1946) suggests that the cotton wilt fungus, *F. vasinfectum*, like *F. culmorum*, is able to colonize dead host tissues in the soil.

The present paper describes an investigation of the ability of four cereal root-rot fungi, *Helminthosporium sativum* P., K. & B. (*Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur, see Tinline, 1951), *Curvularia ramosa* (Bainier) Boedijn, *Ophiobolus graminis* Sacc. and *Fusarium culmorum* (W.G.Sm.) Sacc. to colonize wheat straw buried in unsterilized soil containing graded amounts of inoculum. Subsequent papers will deal with factors influencing saprophytic colonization and with the saprophytic survival of three of these fungi in the soil.

SAPROPHYTIC COLONIZATION EXPERIMENTS

Experimental methods

Development of a suitable technique

The technique for assessing competitive saprophytic ability in the test fungi must ensure proof or disproof of saprophytism under natural soil conditions. Demonstrations that a fungus will not grow through soil from a food-base of agar or other inoculum, in the manner of *Corticium solani* (Blair, 1943), do not prove that it is unable to colonize dead plant tissues; *Fusarium culmorum*, which is a vigorous colonizer of buried wheat straw (Sadasivan, 1939), will not grow out from a food-base in this way (Garrett, 1944). To test the competitive saprophytic ability of any given root-infecting fungus, it is necessary to present it with a suitable substrate, such as the dead tissues of a wholly susceptible host plant (Garrett, 1950). As the four fungi to be studied were all parasitic on wheat, wheat straw was employed in these experiments. The nutrient value of the straw was varied by steeping in solutions of dextrose and/or sodium nitrate, partly because such treatments would make the straw more comparable to wheat tissues killed at an earlier stage of development, when concentrations of soluble carbohydrate and nitrogenous substances would be higher than in the old straw.

Assuming that more inoculum is required for fungi of weak colonizing ability, the method of testing measured the colonizing ability of the fungus against the competition of the microflora of a standard loam soil. The fungus culture on a maize meal-sand medium was progressively diluted with the standard loam soil (unsterilized). Fifty test pieces of wheat straw were buried in each container holding one of this series of inoculum/soil mixtures; the percentage of straws colonized by the test fungus decreased as the proportion of inoculum decreased and that of soil increased. *The rate of this decrease varied inversely as the saprophytic colonizing ability of the test fungus*, so permitting a comparison of saprophytic colonizing ability between the four fungi.

For assessing percentage of straws colonized three methods were compared: (1) isolation on agar plates, (2) Garrett's (1938*b*) wheat-seedling test and (3) incubation on moist sand, to promote sporulation (the 'sand-plate' test). The agar-plate test was not reliable, because the test fungus was often suppressed by more vigorous competitors; the wheat-seedling test was reliable but laborious, and for work with *Helminthosporium sativum* and *Curvularia ramosa*, which spore freely under moist conditions, was superseded by the sand-plate method.

Detailed description of the technique

Preparation of maizemeal-sand inoculum. The maizemeal-sand culture medium comprised: 100 parts sand, 3 parts ground maizemeal and 13 parts water (by weight). After sterilization, each half-litre flask of medium was inoculated with an 8 mm. agar disk of the test fungus and incubated for 1 month at 25° C. The flasks were shaken after 14 days, but otherwise remained undisturbed during the incubation period.

Preparation of straws. From whole lengths of wheat straw, pieces were cut out from immediately below to approximately 1 in. above each node. Subsequently, different lots of straws were treated respectively with (a) distilled water, (b) 2% dextrose solution, (c) 1% sodium nitrate solution and (d) a solution containing 2% dextrose and 1% sodium nitrate. Thorough wetting was ensured by soaking under reduced pressure for 2 hr. Each lot of straws was drained, steamed in 1 l. flasks for 1 hr., and autoclaved 1½ hr. at 1½ atm. Some experiments included unsterilized straws, which received only a 2 hr. soak in distilled water under reduced pressure.

Preparation of soil. The soil used was Kettering loam, passed through a 2 mm. sieve and adjusted to a moisture content equivalent to 45% saturation capacity (Keen & Raczkowski, 1921).

Method of setting up experiments. At the end of the month's incubation inoculum was added to soil in the following proportions: 100 : 0 (inoculum control), 98 : 2, 90 : 10, 50 : 50, 10 : 90, 2 : 98 and 0 : 100 (soil control). Immediately after mixing inoculum and soil, straws (fifty per 200 g. of inoculated soil) were evenly distributed throughout the mixtures, which were then placed in 1 lb. glass jam jars, previously adjusted to a constant weight with clean, fine gravel. Soil controls were similarly treated, but in the inoculum controls straws were added to pure inoculum in 350 ml. conical flasks. As the average weight of the fifty straws added to each weighted jar had been determined previously, it was possible, by tri-weekly waterings, to maintain the mixtures at a constant moisture content throughout the subsequent 4-week incubation period, when the jars were kept at room temperature (16–22° C.).

At the end of this period, straws were washed free of adhering soil and inoculum; saprophytic colonization by the particular test fungus was assessed by one or more of the three methods described below.

*Methods of assessing saprophytic colonization**Agar-plate method*

Straws were surface-sterilized (see below) in 1 : 1000 mercuric chloride in 10% alcohol, rinsed four times in sterile water and plated out, five per petri dish, on acidified potato dextrose agar (pH 5). The plated straws were incubated at 25° C. for 3 weeks for *Helminthosporium sativum* and *Curvularia ramosa*, or 5 days for *Fusarium culmorum*, and the percentage straws yielding the test fungus was recorded.

To secure results by the agar-plate method, surface sterilizing of the straws before plating out was essential; otherwise colonies of the test fungus on the agar plates might be derived from particles of the original maize meal-sand inoculum. Treatment with 1 : 1000 mercuric chloride in 10% alcohol for 1½, 2¼ and 2½ min. destroyed purely external mycelium of *Helminthosporium sativum*, *Curvularia ramosa* and *Fusarium culmorum*, respectively. Colonization by *Ophiobolus graminis* was not assessed by this method, which is unsatisfactory for the isolation of slow-growing fungi.

Seedling infection method

This method was devised by Garrett (1938*b*). A wheat seed was placed, germ end downwards, within the lumen of each piece of straw; the fifty straws with their contained wheat seeds were planted in a seed tray 14 × 8½ × 3 in., filled with sand. Seedlings were grown in the glasshouse and harvested 1 month after planting, and the roots scored for infection. Results were recorded both as percentage infected seedlings, and by a disease rating, based on the severity of seedling infection. The degree of correlation between percentage colonization and colonization rating was sufficiently high to permit the expression of all results as percentage number of straws colonized.

Sand-plate method

Straws were surface-sterilized as in the agar-plate method, and plated out in Petri dishes, containing 50 g. of sterilized sand, moistened with 10 ml. of sterile distilled water. The plates were incubated at 25° C. for 15 days, and the number of straws yielding sporulating colonies of the test fungus, together with the extent of sporulation on individual straws, was recorded. As with the seedling infection method, the correlation between the percentage colonization and the colonization rating figures was high, so the results are expressed only as percentage number of straws colonized.

*Results of saprophytic colonization experiments**Experiments with Fusarium culmorum*

Exp. 1: agar-plate method. Sterilized straws were pretreated with distilled water, dextrose, sodium nitrate and dextrose plus sodium nitrate, and incubated in each of the five standard inoculum/soil mixtures and in pure inoculum and pure soil, respectively; percentages straws colonized are given in Table 1.

TABLE 1. *Colonization of straws by Fusarium culmorum. Agar-plate method*

		Percentage colonized			
		O	D	N	D+N
Inoculum	100 %	100	100	100	100
	98 %	92	88	96	100
	90 %	92	88	100	96
	50 %	92	72	92	48
	10 %	44	56	40	44
	2 %	40	24	32	36
Soil	100 %	4	0	20	4

In this and subsequent tables O, D, N and D+N refer to pretreatments of the straws with distilled water, dextrose, sodium nitrate and dextrose plus sodium nitrate, respectively.

Seedling infection method. From the same experiment, further lots of fifty straws from each of the twenty-eight series were tested for saprophytic colonization by the seedling infection method; percentages straws colonized are given in Table 2.

TABLE 2. *Colonization of straws by Fusarium culmorum. Seedling infection method*

		Percentage colonized			
		O	D	N	D+N
Inoculum	100 %	90	96	90	94
	98 %	87	90	80	76
	90 %	80	90	62	65
	50 %	67	72	60	57
	10 %	65	63	57	58
	2 %	55	60	42	62
Soil	100 %	22	29	23	25

These results show that *Fusarium culmorum* is able to colonize wheat straw in competition with the natural microflora of the soil. The figure of 20–30% test straws colonized in the soil controls proves that *F. culmorum* is widely present in the soil; this confirms the findings of Sadasivan (1939) and Walker (1941) and explains the frequent occurrence of this fungus as a post-harvest colonizer of cereal stubble (Simmonds, 1928; Samuel & Greaney, 1937; and White, 1945).

Experiments with Ophiobolus graminis

Exp. 2: seedling infection method. Sterilized straws, pretreated as in Exp. 1, and also unsterilized straws, were incubated in lots of fifty in each of the five standard inoculum/soil mixtures and in pure inoculum and pure soil, respectively. In addition to the standard mixing technique, in which straws were buried in the inoculum/soil mixtures immediately after mixing, a duplicate series of straws was added to the inoculum/soil mixtures after 24 hr. The percentages straws colonized are given in Table 3.

TABLE 3. Colonization of straws by *Ophiobolus graminis*.
Seedling infection method

		Percentage colonized									
		Straws added immediately					Straws added after 24 hr.				
		O	D	N	D+N	U	O	D	N	D+N	U
Inoculum	100 %	98	100	100	98	87	—	—	—	—	—
	98 %	46	12	76	79	37	13	4	71	75	23
	90 %	8	4	43	54	2	0	0	44	37	0
	50 %	10	2	10	13	4	2	0	4	8	2
	10 %	2	0	0	2	0	0	0	0	0	0
	2 %	0	0	0	0	0	0	0	0	0	0
Soil	100 %	0	0	0	0	0	—	—	—	—	—

U indicates unsterilized straws.

Table 3 shows that *Ophiobolus graminis* has only a limited ability to colonize wheat straw saprophytically in competition with the microflora of the soil. This confirms Garrett's (1950) view that this fungus is a specialized parasite with restricted saprophytic activity. The few dextrose-treated straws yielding *O. graminis*, even at the lowest dilution of inoculum, indicate increased colonization of these straws by saprophytic sugar fungi. This effect was much greater than that due to the natural microflora of the straw, which is shown by a comparison of sterilized (distilled water series) with unsterilized straws. On the other hand, nitrogen pretreatment of the straws increased colonization, indicating that nitrogen is a scarce nutrient in competitive colonization of wheat-straw tissues. Garrett (1940) ascribed the prolonged saprophytic survival of *O. graminis*, in the presence of assimilable nitrogen, to a similar effect.

As expected, the effect of adding the straws to the inoculum/soil mixtures after 24 hr. was to reduce colonization, because of the increased activity of the competitive soil microflora.

Exp. 3. This experiment explored the effect on colonization by *O. graminis* of initially coating the test straws, (a) with inoculum and (b) with soil. Separate lots of fifty straws—sterilized in distilled water and unsterilized, but presoaked in distilled water—were treated in these two ways and buried in inoculum/soil mixtures containing 98, 50 and 2% inoculum, respectively. As a check on the results obtained in *Exp. 2*, a third series (c) of sterilized and unsterilized straws was incorporated in the same inoculum/soil mixtures immediately after mixing. Pure inoculum and pure soil controls were included. The results of this experiment are given in Table 4.

These results emphasize the increased colonization by *O. graminis* of the straws coated initially with inoculum. This demonstrates the great advantage given to *O. graminis* by first contact with the wet, adhesive surface of the straw substrate.

The marked decline in colonization by *O. graminis* of those straws coated initially with soil may conversely be attributed to the comparable advantage thereby given to other soil micro-organisms.

TABLE 4. *Colonization of straws by Ophiobolus graminis*
Seedling infection method

		Percentage colonized					
		Sterilized straws			Unsterilized straws		
		A	B	C	A	B	C
Inoculum	100 %	100	—	—	84	—	—
	98 %	92	49	96	74	24	53
	50 %	61	8	19	38	10	15
	2 %	12	0	0	4	0	0
Soil	100 %	—	0	—	—	0	—

A, B and C indicate straws coated with inoculum, unsterilized soil and inoculum/soil mixture, respectively.

Exp. 4. Separate lots of fifty straws, pretreated as in *Exp. 1*, were coated with inoculum before incubation in each of the five standard inoculum/soil mixtures and in 100 % soil and 100 % inoculum, respectively. The test straws were allowed to stand in contact with the inoculum for approximately 2 hr. before adding the requisite amount of soil in each treatment and mixing straws, inoculum and soil together. Percentages straws colonized are given in Table 5.

TABLE 5. *Colonization of straws by Ophiobolus graminis*.
Seedling infection method

		Percentage colonized			
		O	D	N	D+N
Inoculum	100 %	100	100	100	100
	98 %	100	100	100	100
	90 %	100	100	100	100
	50 %	100	100	100	100
	10 %	80	94	74	92
	2 %	31	45	26	27
Soil	100 %	0	0	0	0

These results confirm those obtained in *Exp. 3* and stress the colonization advantage enjoyed by the test fungus as a result of 2 hr. initial contact with the substrate. At the two highest dilutions of inoculum, colonization appears to have been promoted by pretreatment of the straws with dextrose. As neither of these dilutions provided sufficient inoculum to cover completely all the test straws, this effect almost certainly reflects the increased 'stickiness' of the dextrose-treated straw surfaces and their consequent capacity to retain fragments of inoculum.

Experiments with Curvularia ramosa

Exp. 5a: agar-plate method. Sterilized straws pretreated as before were incubated, in lots of twenty-five, in each of the five standard inoculum/soil mixtures and in pure inoculum and pure soil, respectively; percentage straws colonized are given in Table 6.

TABLE 6. *Colonization of straws by Curvularia ramosa. Agar-plate method*

		Percentage colonized			
		O	D	N	D+N
Inoculum	100 %	100	100	100	100
	98 %	96	76	100	100
	90 %	92	72	88	92
	50 %	76	60	68	96
	10 %	68	32	60	68
	2 %	60	44	64	36
Soil	100 %	0	0	0	0

Exp. 5b: seedling infection method. From the same twenty-eight series, further lots of fifty straws were tested for saprophytic colonization by the seedling infection method; percentages straws colonized are given in Table 7.

TABLE 7. *Colonization of straws by Curvularia ramosa. Seedling infection method*

		Percentage colonized			
		O	D	N	D+N
Inoculum	100 %	100	100	100	100
	98 %	96	100	100	96
	90 %	96	98	94	90
	50 %	96	96	98	98
	10 %	85	100	92	92
	2 %	88	86	81	84
Soil	100 %	0	0	0	0

Table 7 shows that, under comparable conditions, *C. ramosa* is an even more vigorous competitive saprophyte than *Fusarium culmorum*. There is good general agreement with Table 6, where the figures are lower, because *Curvularia ramosa* tends to be overrun by more vigorous competitors on agar plates. As shown in Table 6, dextrose pretreatment of the straws appears to have depressed colonization; as with *Fusarium culmorum*, this is considered to be partly an artefact of the agar-plate method, resulting from the selectivity of the isolation medium for rapid-growing sugar fungi.

Exp. 6: sand-plate method. This experiment investigated the effect on saprophytic colonization by *Curvularia ramosa* of prolonging the period between preparation of the inoculum/soil mixtures and incorporation of the straws. Separate lots of fifty straws—sterilized in distilled water, and unsterilized, but presoaked in distilled

water—were buried immediately in 200 g. lots of bulk-prepared inoculum/soil mixtures, containing 90, 50 and 2% inoculum, respectively. To similar amounts of the same bulk mixtures, placed in jars after mixing, sterilized and unsterilized straws were added at intervals of 1, 3 and 7 days. Pure inoculum and pure soil controls were included. Percentages straws colonized are given in Table 8.

TABLE 8. *Colonization of straws by Curvularia ramosa. Sand-plate method*

No. of days' incubation of soil and inoculum before adding straws			Percentage colonized							
			Sterilized straws				Unsterilized straws			
			0	1	3	7	0	1	3	7
Inoculum	100%	...	100	—	—	—	100	—	—	—
	90%	...	100	76	100	100	100	98	100	100
	50%	...	100	94	100	100	100	100	100	100
	2%	...	88	88	98	100	98	100	100	100
Soil	100%	...	0	—	—	—	0	—	—	—

Results in Table 8 suggest that *C. ramosa* is capable of multiplication in the soil. Thus, at the 2% inoculum level, there is a consistent increase in colonization with increase in inoculum/soil conditioning periods. Though a 24 hr. conditioning reduced colonization in the sterilized straw series at the highest inoculum concentration, longer conditioning periods gave 100% colonization. The temporary or fungistatic nature of this decline in the colonizing activity of *C. ramosa* may be associated with an initial 'flare-up' of the soil microflora on the unconsumed nutrients of the inoculum. The high colonization percentages recorded for unsterilized straws at all inoculum levels indicate that, under natural field conditions, the survival and dispersal of *C. ramosa* may be quite independent of the presence of the living host plant.

Experiments with Helminthosporium sativum

Exp. 7a: agar-plate method. Sterilized straws, pretreated as in Exp. 1, were incubated, in lots of twenty-five, in each of the five standard inoculum/soil mixtures and in pure inoculum and pure soil, respectively. Percentages straws colonized are given in Table 9.

Exp. 7b: seedling infection method. Further lots of fifty straws from each of the twenty-eight series were tested for saprophytic colonization by this method; percentages straws colonized are given in Table 10.

These results show that *H. sativum* can colonize wheat straw, in competition with the soil microflora, only to a limited extent.

Although the seedling infection method proved much more selective for the test fungus than the agar-plate method, the results of both methods agree. Thus, in

competition with saprophytes, *H. sativum* developed on the agar plates, with only one exception, from not more than 12% of any of the eight series of straws incubated at the two highest inoculum levels. Again, with but one exception, it failed to develop from straws incubated at the three lowest inoculum levels. The data suggest that colonization by *H. sativum* is favoured by the dextrose plus nitrate pretreatment of the straw, but this may be an effect on colonization of the agar plate from the straw rather than an effect on original colonization of the straw from the inoculum/soil mixtures.

TABLE 9. Colonization of straws by *Helminthosporium sativum*. Agar-plate method

		Percentage colonized			
		O	D	N	D+N
Inoculum	100 %	92	92	96	100
	98 %	12	8	8	72
	90 %	4	4	4	12
	50 %	0	0	0	8
	10 %	0	0	0	0
	2 %	0	0	0	0
Soil	100 %	0	0	0	0

TABLE 10. Colonization of straws by *Helminthosporium sativum*. Seedling infection method

		Percentage colonized			
		O	D	N	D+N
Inoculum	100 %	100	100	96	100
	98 %	33	30	10	16
	90 %	46	39	17	36
	50 %	10	21	8	29
	10 %	8	10	6	10
	2 %	2	0	0	0
Soil	100 %	0	0	0	0

On the results of the seedling infection method, however, it appears that nitrogen pretreatment effects a reduction in colonization; this effect is confirmed by the results of the next experiment.

Exp. 8: sand-plate method. Sterilized straws, pretreated as in Exp. 1, and untreated, unsterilized straws were incubated, in lots of fifty, in each of the five standard inoculum/soil mixtures and in pure inoculum and pure soil, respectively. In addition to the standard mixing technique, in which straws were buried in the various inoculum/soil mixtures immediately after mixing, a duplicate series of straws was added to the same mixtures after a time lag of 24 hr. Percentages straws colonized are given in Table 11.

The results of this experiment are in general agreement with those shown in Table 10. As in the preceding experiment, nitrogen pretreatment of the straws

tended to depress colonization by *H. sativum*. This effect may be due to an increase in colonization of the nitrogen-enriched straws by other members of the soil microflora, many of which can use nitrate nitrogen (Foster, 1949; Lilly & Barnett, 1951). It is not due to the inability of *H. sativum* to utilize nitrate nitrogen, as this possibility has been excluded by experiment (to be described in the third paper in this series).

TABLE 11. *Colonization of straws by Helminthosporium sativum. Sand-plate method*

		Percentage colonized									
		Straws added immediately					Straws added after 24 hr.				
		O	D	N	D+N	U	O	D	N	D+N	U
Inoculum	100 %	100	100	86	98	2	—	—	—	—	—
	98 %	0	0	2	0	0	56	70	18	64	2
	90 %	60	52	30	40	58	50	42	30	34	34
	50 %	38	40	26	36	26	42	36	28	34	12
	10 %	0	2	0	0	0	0	0	0	2	0
	2 %	0	0	0	0	0	0	0	0	0	0
Soil	100 %	0	0		0	0	—	—	—	—	—

Table 11 also shows that, in contradistinction to *Curvularia ramosa*, *Helminthosporium sativum* failed to establish itself, to any appreciable extent, in unsterilized straws incubated either in 98% or in 100% inoculum, though considerable colonization occurred in 90% and in 50% inoculum. This result, together with an anomalous failure to colonize sterilized straws in one series of 98% inoculum, suggests that biological competition is not the sole factor influencing saprophytic colonization by *H. sativum*.

Exp. 9: sand-plate method. This experiment was made to find the respective saprophytic colonizing ability of the Australian isolate of *H. sativum*, hitherto used exclusively in this investigation, and of a Canadian isolate, supplied by Dr P. M. Simmonds.

For each isolate, four series of straws, previously sterilized in distilled water, were incubated, in lots of fifty, in inoculum/soil mixtures containing 98, 50 and 10% inoculum, respectively. Two of these four series of straws were added immediately to the inoculum/soil mixtures; the remaining two were added 24 hr. after mixing inoculum and soil. Subsequently, fifty incubated straws from each 100 thus far given similar treatment were dried at 27° C. for 12 hr., after standard surface sterilization with mercuric chloride, and then plated out on moist, sterile sand; the remaining fifty were plated out wet immediately after surface sterilization. Duplicate lots of straws were incubated in pure inoculum of each of the isolates, and also in pure soil, and were planted out dry and wet, respectively. Results of this experiment are given in Table 12.

The colonization results obtained with both isolates are in general agreement with those of the preceding experiments. Drying of the straws before sand plating did

not increase the number of *H. sativum* colonies, as had been expected. The anomalously low degree of colonization in 98% inoculum (at zero hour mixing of straws with inoculum/soil), obtained in Exp. 8 (Table 11), did not appear again in the present experiment.

TABLE 12. Colonization of straws by *Helminthosporium sativum*.
Sand-plate method

				Percentage colonized							
				Australian isolate				Canadian isolate			
Plated dry or wet				Dry		Wet		Dry		Wet	
Added to inoculum/soil mixtures after				0 hr.	24 hr.	0 hr.	24 hr.	0 hr.	24 hr.	0 hr.	24 hr.
Inoculum	{	100 %		100	—	100	—	100	—	100	—
		98 %		94	86	86	84	88	90	86	86
		50 %		44	40	52	42	42	36	34	32
		10 %		10	10	12	14	4	6	4	10
Soil		100 %		0	—	0	—	0	—	0	—

DISCUSSION

The data presented show that *Fusarium culmorum* and *Curvularia ramosa* behave as vigorous saprophytic colonizers of wheat straw buried in unsterilized soil, whereas saprophytic colonization of straw tissues by *Ophiobolus graminis* and *Helminthosporium sativum* is strictly limited in the presence of the soil microflora. Under comparable conditions, *Curvularia ramosa* proved to be an even more vigorous saprophytic colonizer of the test substrate than *Fusarium culmorum* and can be assigned, therefore, to that group of unspecialized root parasites designated by Garrett (1950) as 'soil-inhabiting fungi'. The competitive saprophytic ability of *Helminthosporium sativum*, on the other hand, was shown to be much more restricted than that of *Ophiobolus graminis*. *Helminthosporium sativum* can no longer be regarded, therefore, as 'a vigorous soil saprophyte capable of persisting without the host' (Sanford, 1946), but rather as a highly specialized member of Garrett's root-inhabiting group of fungi. However, by comparison with *Ophiobolus graminis*, *Helminthosporium sativum* is restricted in its parasitic activity, as shown by a limitation to seedling hosts (Stakman, 1920; Broadfoot, 1933) and to plants predisposed to attack by frost injury (Hynes, 1938; Butler, 1948) or other cause (Stakman, 1920; Dosdall, 1923; Hynes, 1938). In this respect this fungus shows a departure from the behaviour of specialized root-infecting fungi as defined by Garrett (1950), since its degree of parasitic specialization is not complementary to its low competitive saprophytic ability.

The general conclusions from this investigation affirm the soundness of Waksman's (1917) original division of soil fungi into general 'soil inhabitants' and more specialized 'soil invaders', and the correctness of his contention that the best

criterion of a general soil saprophyte is that of a widespread and general distribution in the soil. Such a soil-inhabiting saprophyte is essentially an unspecialized fungus, with ability for colonization of a wide variety of dead plant tissues. Conversely, with increasing specialization of substrate, the distribution of a fungus in the soil must become inevitably more local and restricted. The type of distribution of a fungus in the soil is thus the first and most reliable guide to its saprophytic behaviour, but the exact definition of its 'ecological niche' in nature must remain as a research problem for each individual fungus.

This investigation was carried out during the tenure of a Walter and Eliza Hall Research Fellowship in Agriculture of the University of Sydney. I wish to express my thanks to Dr S. D. Garrett for much valuable advice and for his continued interest in the investigation; to Prof. W. L. Waterhouse, Dr C. R. Millikan and Dr P. M. Simmonds for supplying cultures of some of the test fungi; and to Mr F. T. N. Elborn for photographic work accompanying the text.

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SAPROPHYTIC BEHAVIOUR OF SOME CEREAL ROOT-ROT FUNGI

II. FACTORS INFLUENCING SAPROPHYTIC COLONIZATION OF WHEAT STRAW

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Factors influencing the saprophytic colonizing ability of two cereal root-rot fungi, *Curvularia ramosa*, a vigorous competitive saprophyte, and *Helminthosporium sativum*, a weak competitive saprophyte, have been investigated. The observed difference in their competitive saprophytic ability could not be ascribed either to a difference in rate of growth or to the ability of the stronger saprophyte, *C. ramosa*, to produce an antibiotic. The weaker saprophyte, *H. sativum*, proved, however, to be more sensitive than *C. ramosa* to antibiotics produced by other soil micro-organisms. Thus, in spore germination tests with purified fungal antibiotics *in vitro*, *H. sativum* proved more sensitive to eight of the ten substances tested than did *C. ramosa* and was only slightly less sensitive than *C. ramosa* to the remaining two antibiotics. *H. sativum* was found also to be generally more sensitive than *C. ramosa* to the antagonistic effects of certain common soil bacteria. Of the seven bacterial species tested, five caused an appreciable reduction in germ tube development and two reduced germination in *H. sativum* whereas none of the bacterial species inhibited either spore germination or germ tube development of *C. ramosa*, and three actually stimulated germ tube development by this fungus.

INTRODUCTION

On the results of saprophytic colonization experiments with cereal root-rot fungi, discussed in the first paper of this series (Butler, 1953), *Curvularia ramosa* (Bainier) Boedijn was classified as a vigorous competitive saprophyte and was assigned to that group of root-disease fungi designated by Garrett (1950) as soil inhabitants. *Helminthosporium sativum* P., K. & B., on the other hand, was found to behave as a weak competitive saprophyte and was included, therefore, in that group of specialized parasites which Garrett has termed root-inhabiting fungi. Since *C. ramosa* and *H. sativum* are similar morphologically and yet differ so strikingly in their behaviour as competitive saprophytes, they were selected for an investigation of factors influencing the saprophytic colonizing ability of root-disease fungi.

Garrett (1950) suggested that the success of a vigorous competitive saprophyte might be correlated, in part at least, with (1) a high growth rate and dense growth habit, (2) ability to produce toxic growth substances, (3) tolerance of antibiotics produced by competing micro-organisms. These possibilities were therefore considered, in turn.

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RELATIVE GROWTH-RATE DETERMINATIONS

Apart from a paper by Hynes (1935) there appear to be no data on the relative growth rates of *C. ramosa* and *H. sativum*. Of thirty-one strains of *H. sativum* tested, Hynes recorded nineteen with colony diameters below 40 mm. after 6 days' incubation at 25° C.; each of eleven strains of *C. ramosa* (*Helminthosporium* M) showed a colony diameter exceeding 40 mm. Colony diameters recorded by Henry (1924) for a single strain of *C. ramosa* (*Helminthosporium* M, strain 1) also exceeded 40 mm. after 6 days' incubation between 19.6 and 32° C. Such data indicated that the higher saprophytic colonizing ability of *C. ramosa* might well be due to its higher growth rate.

Pilot colonies of *C. ramosa* and *H. sativum* were grown on potato dextrose agar plates for 5 days at 25° C.; from these colonies, 4 mm. inoculum disks were taken from the growing margin to inoculate twelve plates of potato dextrose agar with each fungus. The plates were incubated in triplicate at 22.5, 25 and 27.5° C., respectively, and at room temperature (range 15–29° C.). Results are given in Table 1.

TABLE 1. *Mean diameter of colonies in mm.*

Test fungus	Incubation temperature (° C.)	Incubation period in hours		
		27	87	152
<i>C. ramosa</i>	22.5	8.5	32.5	60.5
	25	11	39	72
	27.5	12	40	73
	Room temp.	7	25	51.5
<i>H. sativum</i>	22.5	10	34	67.5
	25	12	39	74
	27.5	11.5	42	73
	Room temp.	9	30.5	56

Table 1 shows that the growth rate of the more vigorous saprophytic colonizer, *C. ramosa*, was generally somewhat lower than that of *H. sativum*. It therefore appears unlikely, from these results, that a difference in growth rate between these two fungi can explain the observed difference in their saprophytic behaviour.

RELATIVE SENSITIVITY OF *CURVULARIA RAMOSA* AND *HELMINTHOSPORIUM SATIVUM* TO PURE ANTIBIOTICS *IN VITRO*

The extensive literature on antibiotics, which has been reviewed by Waksman (1945, 1948), Brian (1949, 1951), Bailey & Cavallito (1948) and Weindling, Katznelson & Beale (1950), shows that they are produced by all groups of micro-organisms. Many investigators have remarked upon the sensitivity of *H. sativum* to the antagonistic effects of other soil micro-organisms. On the other hand, there appear to be no data in the literature concerning the sensitivity of *C. ramosa* to the competitive and antibiotic effects of other micro-organisms.

Observations during the saprophytic colonization experiments (Butler, 1953)

suggested that *C. ramosa* was generally less sensitive to antibiotic effects than was *H. sativum*. Thus, when colonization by these two fungi was assessed by the agar-plate method, *C. ramosa* usually appeared as distinct colonies, but *H. sativum* produced only occasional conidiophores. It was decided, therefore, to test the action of certain antibiotics *in vitro* on spore germination of the two test fungi.

The antibiotics selected were aurantiogliocladin, frequentin, gliotoxin, griseofulvin, mycophenolic acid, patulin, rubrogliocladin, trichothecin, viridin and ustilagic acid. With the exception of ustilagic acid, all these antibiotics are produced either by fungi recorded as wheat-straw colonizers in association with the test fungi or by species commonly isolated from soil.

The fungicidal and fungistatic effects of the various test antibiotics were assayed by a slightly modified version of the spore germination technique of Brian & Hemming (1945). The only variations from this technique (used with *Botrytis allii*) were in the age of test cultures of *C. ramosa* and *H. sativum*, the density of the test spore suspensions and, as mentioned later, the criteria adopted to assess the sensitivity of the test fungi. For both *C. ramosa* and *H. sativum* it was found desirable to use 7–10 days old cultures and to adjust the spore concentrations to 250,000–300,000 spores per ml. The water-soluble antibiotics, aurantiogliocladin, patulin, rubrogliocladin and ustilagic acid, were made up immediately in the required concentrations in distilled water: frequentin, gliotoxin, griseofulvin, mycophenolic acid and trichothecin were first dissolved in a small quantity of absolute alcohol, and viridin was taken up initially in a small quantity of dilute sodium hydroxide solution. Equivalent quantities of the solvents used for the water-insoluble antibiotics were added to the control spore suspensions in each case. Spore suspensions of each test fungus were then made up in concentrations ranging from 200 parts per million (water-soluble antibiotics) or 100 p.p.m. (water-insoluble antibiotics) to 1 p.p.m. Subsequently, three individual drops of spore suspension in each concentration of antibiotic were placed on a sterile glass slide in a Petri-dish moist chamber. The drops were incubated for 8 to 10 hr. at 25° C. and then stained with dilute cotton blue in lactophenol. Following Brian & Hemming (1945), a preliminary assessment of spore germination in each drop was made according to the categories: 0=no germination; 1=1–5%; 2=5–50%; 3=50–90% and 4=90–100% germination. Except in two experiments that had to be repeated on account of an error in technique (see below), all three drops per slide showed similar germination and so a permanent preparation was made of the central drop only. Subsequently, the percentages were determined more accurately by examining 300 individual spores and the mean length of germ tubes at each concentration of antibiotic was determined from fifty measurements. Counts and measurements on a greater number of spores were shown to be unnecessary. These two criteria of sensitivity of the test fungi to antibiotics were adopted when it was found that germ-tube development was inhibited at lower concentrations than spore germination.

The results obtained with each of the ten antibiotics tested are presented in summary form. Table 2 records only the least concentrations of each antibiotic found to reduce spore germination and germ tube development by more than 10% and 20%, respectively. The abnormal 'curling' effect of griseofulvin (Brian, Curtis & Hemming, 1949) on *H. sativum*, and, to a lesser extent, on *C. ramosa* prevented an accurate assessment of germ tube length, and a 'curling' effect rating was therefore substituted.

TABLE 2. *Least concentrations of antibiotics (p.p.m.) required to cause 10% reduction in spore germination and 20% reduction in germ-tube length*

Antibiotic	<i>H. sativum</i>		<i>C. ramosa</i>	
	Germination	Germ tubes	Germination	Germ tubes
<i>Antibiotics to which Helminthosporium sativum is more sensitive than Curvularia ramosa</i>				
Aurantogliocladin	50	12	200	100
Griseofulvin	> 100	2*	> 100	6*
Mycophenolic acid	50	6	> 100	> 100
Patulin	50	6	200	25
Rubrogliocladin	50	25	> 200	> 200
Trichothecin	6	< 1	12	3
Ustilagic acid	> 200	12	> 200	100
Viridin	2	< 1	12	2
<i>Antibiotics to which Curvularia ramosa is more sensitive than Helminthosporium sativum</i>				
Frequentin	50	3	25	3
Gliotoxin	25	6	12	3

* Least concentration producing 'curling' effect.

As shown in Table 2, aurantiogliocladin, griseofulvin, mycophenolic acid, patulin, rubrogliocladin, trichothecin, ustilagic acid and viridin were more fungistatic, though in varying degree, to *H. sativum* than to *C. ramosa*. Frequentin and gliotoxin were somewhat more fungistatic to *C. ramosa* than to *H. sativum*. Germ-tube development of *H. sativum* was depressed by all antibiotics at concentrations of 25 p.p.m. or less, whereas mycophenolic acid and rubrogliocladin were not fungistatic to *C. ramosa* even at the maximum test concentrations, and aurantiogliocladin and ustilagic acid proved fungistatic only at a concentration of 100 p.p.m.

These results, therefore, support the hypothesis that the low competitive saprophytic ability of *H. sativum* is due to its sensitivity to antibiotic effects; conversely, the vigorous saprophytic behaviour of *C. ramosa* reflects its general tolerance of antibiotics produced by fungal competitors. Viridin and trichothecin, the most fungistatic antibiotics tested, are produced, respectively, by *Trichoderma viride* and *Trichothecium roseum*, both frequent wheat-straw colonizers.

In two earlier tests with viridin and mycophenolic acid, spore germination did not exceed 2% in any series, including the controls. These abnormal results were subsequently shown to be due to the addition of water to dry, but previously used, filter-papers in the Petri dish moist chambers, some 36 hr. before their use in the

viridin and mycophenolic acid tests. A similar effect was noted by Brown (1922) who attributed such spore germination failures to the production of volatile substances by micro-organisms growing on wet filter-paper. Following the results of the initial experiments with viridin and mycophenolic acid, new, freshly moistened filter-papers were used in all subsequent antibiotic tests.

RELATIVE SENSITIVITY OF *CURVULARIA RAMOSA* AND *HELMINTHOSPORIUM SATIVUM* TO CERTAIN SOIL-BORNE BACTERIA

In tests with *H. sativum*, rubrogliocladin proved fungistatic to this fungus at concentrations down to 25 p.p.m.; germ-tube development was normal at three lower concentrations but was checked at 2 and 1 p.p.m. This check was subsequently found to be due to contamination with a bacterium (in form a coccus, but otherwise unidentified). This effect suggested an exploration of the sensitivity of *H. sativum* and *C. ramosa* to common soil-inhabiting bacteria.

The bacterial species selected were: *Bacillus licheniformis* (Weigmann) Chester, *B. mesentericus* (Flügge) Migula, *B. mycoides* Flügge, *B. polymyxa* (Prazmowski) Migula, *B. subtilis* Cohn emend. Prazmowski, *Pseudomonas aeruginosa* (Schroeter) Migula and *Ps. fluorescens* Migula.

Of these common soil inhabitants, *B. mycoides* and *B. subtilis* had previously been recorded as wheat straw colonizers, as had species identical with or closely related to *B. mesentericus* and *Ps. fluorescens*.

Spore germination drops of *H. sativum* and *C. ramosa*, inoculated with the respective bacteria, together with uninoculated control drops, were recorded after incubation at 25° C. for 8 hr. (see Table 3).

TABLE 3. *Effects of certain soil-borne bacteria on spore germination and germ tube development of Helminthosporium sativum and Curvularia ramosa*

Bacteria	<i>H. sativum</i>		<i>C. ramosa</i>	
	% spore germination	Mean length of germ tubes in μ	% spore germination	Mean length of germ tubes in μ
<i>B. subtilis</i>	94	157	100	246
<i>Ps. fluorescens</i>	96	191	99	177
<i>B. mycoides</i>	96	186	100	151
<i>B. mesentericus</i>	52	38	98	167
<i>B. polymyxa</i>	95	248	98	231
<i>B. licheniformis</i>	98	228	99	230
<i>Ps. aeruginosa</i>	85	169	99	143
Control	96	256	100	145

All bacteria tested, except *B. polymyxa* and *B. licheniformis*, markedly reduced the development of germ tubes of *H. sativum*. In addition, *B. mesentericus* and, to a lesser extent, *Ps. aeruginosa*, reduced spore germination of this fungus. On the other hand, neither spore germination nor germ-tube development of *C. ramosa* was suppressed by any of the test bacteria; indeed, germ-tube development

of this fungus was actually stimulated by *B. subtilis*, *B. polymyxa* and *B. licheniformis*.

These results indicate that *H. sativum* is generally more sensitive to the antagonistic effects of soil-inhabiting bacteria than is *C. ramosa*, thus supporting the hypothesis that the contrasting saprophytic behaviour of *H. sativum* and *C. ramosa* is largely a reflexion of their relative sensitivity to antagonistic effects in the soil.

The marked sensitivity of *H. sativum* to *B. mesentericus*, which suppressed both germination and germ-tube growth to a greater extent than any other test bacterium, has been noted previously by Christensen & Davies (1940). The increased development of germ tubes of *C. ramosa* in association with *B. subtilis* may be due to the production of glutamic acid by this bacterium (Bovarnick, 1942) and its subsequent utilization by *C. ramosa*. Srivastava (1950) found that *C. lunata* grew best on a synthetic medium in which glutamic acid (or asparagine) was substituted for potassium nitrate. Since many bacteria produce not only amino-acids but also protein, polypeptide and polysaccharide substances (Stephenson, 1949), the production of such substances by *B. polymyxa* and *B. licheniformis* may be responsible for their stimulatory effect on *C. ramosa*.

SCREENING TESTS FOR PRODUCTION OF ANTIBIOTIC SUBSTANCES BY *CURVULARIA RAMOSA* AND *HELMINTHOSPORIUM SATIVUM*

Most fungal antibiotics are produced by soil-inhabiting saprophytes; relatively few have been isolated from plant pathogenic fungi and none from either *C. ramosa* or *H. sativum*. However, Ludwig (1950) showed that *H. sativum* produced a phytotoxic substance, Carter (1935) suggested that it inhibited a bacterium in culture, and Brömmelhues (1935) found that it was antagonistic to *Ophiobolus graminis*.

Through the courtesy of Dr P. W. Brian, preliminary tests on the antibiotic activity of isolates of *C. ramosa* and *H. sativum* were made in the Butterwick laboratories of Imperial Chemical Industries Ltd. In these tests (Brian, 1952), *C. ramosa* showed 'virtually no antifungal or antibacterial activity', whereas both the Australian and the Canadian isolates of *H. sativum* yielded antifungal culture filtrates. The Canadian isolate also showed 'definite though not very great activity against *B. subtilis*'.

The inability of the more vigorous saprophyte, *C. ramosa*, to produce an antibiotic substance indirectly supports the hypothesis that a marked tolerance of antibiotics produced by other micro-organisms is the major reason for the success of this fungus as a competitive saprophyte.

This investigation was carried out during the tenure of a Walter and Eliza Hall Research Fellowship in Agriculture of the University of Sydney. I wish to express my thanks to Dr S. D. Garrett for much valuable advice and for his continued interest in the investigation; to Dr P. W. Brian for supplying antibiotics and

arranging for the antibiotic activity of *Curvularia ramosa* and *Helminthosporium sativum* to be tested; and to Dr R. H. Haskins for supplying a test sample of ustilagic acid.

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SAPROPHYTIC BEHAVIOUR OF SOME CEREAL ROOT-ROT FUNGI

III. SAPROPHYTIC SURVIVAL IN WHEAT STRAW BURIED IN SOIL

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Records are given of the survival of *Helminthosporium sativum*, *Curvularia ramosa* and *Ophiobolus graminis* as saprophytes in infected wheat straw buried in unsterilized soil of different nitrate nitrogen content. Ample nitrogen supply prolonged the survival of *O. graminis* and *C. ramosa* but shortened that of *H. sativum*. Both the Australian isolate of *H. sativum* used for the survival experiment and a Canadian isolate of the fungus were able to utilize nitrate, as well as ammonium and peptone nitrogen; neither isolate required either thiamin or biotin for growth in pure culture. The depressing effect of nitrate nitrogen upon survival of *H. sativum* is ascribed to its encouragement of competing micro-organisms also present in the infected straws.

INTRODUCTION

Although it has long been established that root and foot-rot diseases of wheat and other cereals are soil-borne, little is known of the saprophytic survival of their causal fungi in infected host tissues in the soil. Essential data on survival in *unsterilized soil* are available only for *Ophiobolus graminis*. Garrett (1938, 1940, 1944) has shown that, given ample nitrogen, this fungus in wheat straw buried in natural soil shows undiminished viability after at least 34 weeks, although viability is lost more rapidly in the absence of adequate nitrogen. Garrett suggested that nitrogen promoted a slow saprophytic development of this fungus within the infected straw.

The only publication on the survival of *Curvularia ramosa* is by Henry (1924) who found that this fungus, and *Helminthosporium sativum*, remained viable for 5 months in wheat-seed culture in sterilized soil and in packets of straw on the surface of unsterilized soil. Christensen (1922) showed that *H. sativum* remained viable for approximately 14 months in artificially infected wheat straw in bottles 'placed outdoors' and in naturally infected wheat and barley straw 'left unprotected in the field' (presumably on the surface). Hynes (1938) buried sealed glass tubes containing spores of *H. sativum* in soil and reported 30% spore viability after 12 months and 3% after 33 months. Katznelson (1940a) inoculated sterilized soil with a spore suspension of *H. sativum*, alone and with two bacteria and with two actinomycetes, and found that the fungus developed only in the absence of the

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bacteria and actinomycetes. From unsterilized soil, inoculated similarly, Katznelson (1940*b*) was unable to reisolate *H. sativum* after 167 days. Anwar (1949) was rarely able to isolate *H. sativum* from 'infested soil' and so concluded that its saprophytic survival in nature is limited.

None of these investigators reports on the survival of *C. ramosa* and *H. sativum* in infected host tissues in competition with the natural soil microflora. For this reason, and to elucidate the problem of the persistence of *H. sativum*, in view of its low competitive saprophytic ability and restricted parasitic activity (Butler, 1953*a, b*), the survival experiments described in this paper were conducted with *H. sativum* and *C. ramosa*. For comparative purposes, a similar experiment was carried out with *Ophiobolus graminis*.

EXPERIMENTAL METHOD

The technique used with all three fungi was similar to that already described by Garrett (1938) for *O. graminis*. Previously sterilized lengths of wheat straw, inoculated with a maize meal-sand culture of the test fungus (in the proportion of 1000 straws to 300 g. culture) were incubated for 1 month at 25° C. The test straws were then removed, washed free of adhering fragments and buried, in lots of fifty, in 200 g. amounts of each of two series of Kettering loam soil. One soil series was enriched with nitrogen (as sodium nitrate) at the rate of 0.5 g. N per 100 g. dry weight of straw; the other series was denitrified before use, as described by Garrett (1940). The final nitrate nitrogen contents of the enriched and the deficient soil series were 22.2 and 0.5 mg. per 100 g. air-dry soil, respectively.

The survival test jars, in which the soil was maintained at 45% saturation, were kept at room temperature throughout the experimental period. For each fungus, 100 straws from two randomly selected jars in each soil series were removed at each of eight samplings. Samples were taken at 2 and 4 weeks, then at 4-weekly intervals up to 28 weeks. On removal, the straws were washed free of soil but were not surface sterilized; those from the *H. sativum* and *C. ramosa* series, however, were dried at 27° C. for 18 hr.

The survival of *O. graminis* was determined by Garrett's (1938) seedling infection method. A wheat grain was inserted in each test straw, and straws plus contained wheat grains were planted in sand. After 4 weeks, the seedlings were harvested and scored for infection. The survival of *H. sativum* and *C. ramosa* was determined by the sand-plate method (Butler, 1953*a*), and the number of straws yielding sporulating colonies of the respective test fungi at each sampling was recorded.

RESULTS

Saprophytic survival of Helminthosporium sativum

The results of this experiment are given in Table 1.

As indicated below, the data for the 2- and 4-weeks samplings were recorded following a partial repetition of this experiment; the original data obtained at these

times of sampling, when the test straws were infested with larvae of a fungus gnat (possibly *Sciara tritici*), are given in Table 2.

Later, many straws in all test series were found to be infested with fungus gnat larvae, which were frequently seen to ingest conidiophores and spores in one colony and then to migrate to another. The treatment adopted for destroying the

TABLE 1. *Percentage survival of Helminthosporium sativum in wheat straw buried in unsterilized soil. Straws plated dry*

	Weeks								
	0	2	4	8	12	16	20	24	28
Nitrogen-enriched soil	100	98*	100*	96	62	62	30	24	33
Nitrogen-deficient soil	100	100*	100*	96	74	78	68	55	50

* Figures recorded following partial repetition of experiment (see text).

TABLE 2. *Percentage survival of Helminthosporium sativum in wheat straw buried in unsterilized soil. Straws plated wet*

	Weeks						
	0	2	4	8	12	16	20
Nitrogen-enriched soil	100	92*	32*	76	32	26	22
Nitrogen-deficient soil	100	88*	25*	72	40	36	32

* Larvae of fungus gnat occurred on test straws (see text).

larvae without affecting the fungus was to dry the straws, after washing free of soil, for 18 hr. at 27° C. As a comparison of Tables 1 and 2 shows, dry plating not only destroyed the contaminant larvae, but also gave a truer assessment of the actual survival of *H. sativum*, and was used both for the remainder of this test and throughout the survival test with *C. ramosa*. The enhanced development of *H. sativum* on the dry-plated straws was probably due to a reduction in the activity of certain surface-inhabiting antagonistic micro-organisms.

The data in Table 1 prove that *H. sativum* can remain viable, *once well established in wheat-straw tissues*, for at least 28 weeks in unsterilized soil. Presumably, therefore, this fungus could survive for a comparable period in wheat tissues infected during the parasitic phase, in which it enters ahead of all competitors. Under Australian conditions, the period between one cereal harvest and the appearance of new potential host plants (sown or volunteer cereals and susceptible grasses), on which *H. sativum* can resume its parasitic phase, is considerably less than 28 weeks. Accordingly, despite the weakness of *H. sativum* as a competitive saprophyte (Butler, 1953*a*), it can survive saprophytically in diseased host tissues from one season to the next.

A comparison of survival data for straws buried in nitrogen-enriched and for those in nitrogen-deficient soil shows that *H. sativum* survived in somewhat fewer straws under conditions of ample nitrogen supply. This reduction in survival

presumably reflects increased competition. On Garrett's (1940) hypothesis that nitrogen promotes longevity of *O. graminis* by encouraging its saprophytic development, the converse effect on survival of *H. sativum* suggests the absence of even such a limited saprophytic activity, and the encouragement of competing micro-organisms.

Nitrogen utilization by Helminthosporium sativum

The following experiment was made to determine if *H. sativum* could utilize nitrate nitrogen. The Australian isolate of *H. sativum* and a Canadian isolate were grown in 180 ml. medicine bottles containing 10 ml. of the basal culture solution alone, and with 0.01 and 0.02% added nitrogen as sodium nitrate, ammonium tartrate and peptone, respectively. The basal solution consisted of 2% dextrose, 0.1% potassium phosphate, 0.05% crystalline magnesium sulphate, ferric chloride (0.2 mg./l.), thiamin 50 µg./l. and biotin 20 µg./l. Each bottle was inoculated with a 4 mm. inoculum disk from the margin of a 6 days old colony on plain water agar. Three bottles were inoculated for each series; after 21 days' incubation at 25° C., the mycelial pads were removed and dried at 80° C. to constant weight. The total weights of the three pads in each treatment which were pooled, being washed, dried and weighed together, are given in Table 3.

TABLE 3. *Mycelial dry weights in mg.*

	Australian isolate	Canadian isolate
No N—control	3	4
0.01 % N as sodium nitrate	221	124
0.02 % N as sodium nitrate	257	141
0.01 % N as ammonium tartrate	169	87
0.02 % N as ammonium tartrate	223	123
0.01 % N as peptone	249	111
0.02 % N as peptone	291	186

These results show that *H. sativum* can utilize nitrate and other sources of nitrogen and thereby confirm reports by Patel & Kulkarni (1949) and by Lilly & Barnett (1951, p. 101) that this fungus is not specific in its nitrogen requirements.

Growth factor requirements of Helminthosporium sativum

In this part of the experiment, the effect of omitting thiamin and biotin, singly and together, from the basal solution was tested. Mycelial dry weights are given in Table 4.

TABLE 4. *Mycelial dry weights in mg.*

	Australian isolate	Canadian isolate
No growth factors	169	77
Thiamin alone	163	68
Biotin alone	176	79
Thiamin plus biotin	160	59

These results show that *H. sativum* does not require either thiamin or biotin for growth.

Saprophytic survival of Curvularia ramosa

The results of this experiment are given in Table 5.

TABLE 5. *Percentage survival of Curvularia ramosa in wheat straw buried in unsterilized soil*

	Weeks								
	0	2	4	8	12	16	20	24	28
Nitrogen-enriched soil	100	100	99	100	100	98	86	89	78
Nitrogen-deficient soil	100	100	100	100	99	95	74	72	59

As a result of dry-plating the test straws throughout this experiment, no difficulty was experienced with fungus gnat larvae. These data show that *C. ramosa*, apart from its marked ability to colonize wheat straw as a saprophyte (Butler, 1953*a*), can also survive saprophytically in straws buried in the soil for at least 28 weeks. After 28 weeks, *C. ramosa* was recovered from approximately twice as many straws as was *H. sativum*. After 20 weeks and more, the survival of *C. ramosa* was somewhat greater in straws from the nitrogen-enriched soil. The contrasting effects of nitrogen on the saprophytic survival of *C. ramosa* and *H. sativum* again illustrate differences in their saprophytic abilities.

Saprophytic survival of Ophiobolus graminis

The results of this experiment are given in Table 6.

TABLE 6. *Percentage survival of Ophiobolus graminis in wheat straw buried in unsterilized soil*

	Weeks								
	0	2	4	8	12	16	20	24	28
Nitrogen-enriched soil	100	97	91	92	92	81	89	53	40
Nitrogen-deficient soil	100	100	93	89	67	33	11	2	3

These data show that in the nitrogen-enriched soil *O. graminis* remained viable in 89% of the test straws for 20 weeks; even after 28 weeks it still survived in 40% of the straws. On the other hand, its viability fell rapidly after 12 weeks in the nitrogen-deficient soil. These findings confirm those of Garrett (1938, 1940, 1944) in his studies on *O. graminis*.

DISCUSSION

Since the saprophytic colonizing ability of both *H. sativum* and *O. graminis* is restricted in the presence of the soil microflora (Butler, 1953*a*), these two fungi can rarely behave as competitive saprophytic colonizers of wheat-plant tissues in the field. Probably, in nature, both persist primarily in infected host tissues. *H. sativum* is an unusual type of specialized root inhabitant, since it appears to be

both a weak competitive saprophyte and a somewhat restricted parasite (Butler, 1953*a*). However, the parasitism of *H. sativum* is restricted only in so far as plants lacking in normal resistance are more susceptible to attack, and the evidence suggests that senescent plants are particularly liable to infection. The presence of this fungus as a dominant in isolations from wheat plants after anthesis (White, 1945) and its common occurrence in wheat-crown platings (Broadfoot, 1934), suggest, therefore, that it is well adapted to enter such senescent tissues and does so in advance of obligate saprophytes. This helps to ensure the survival of *H. sativum* from one host crop to the next.

C. ramosa proved better able to survive saprophytically than did either *H. sativum* or *O. graminis*. Further, the vigorous competitive saprophytic behaviour of *C. ramosa* (Butler, 1953*a*) would seem sufficient to ensure its persistence in nature. *C. ramosa* is also capable of at least a temporary increase in unsterilized soil (Butler, 1953*a*), and may prove to be a free-living saprophyte in Australian wheat soils.

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SOME EFFECTS OF TEMPERATURE AND NITROGEN SUPPLY ON WHEAT POWDERY MILDEW

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(With 6 Text-figures)

The wheat variety Red Standard was more susceptible to infection by *Erysiphe graminis* at 14–20° C. than at *c.* 7° C. Conidia were produced per unit area of pustule more than ten times as rapidly at 14° C. as at 7° C.

The increased susceptibility of wheat to mildew after applying nitrogenous fertilizer (N) was associated with changes in its growth rate. Both growth rate and susceptibility increased to a maximum and then declined; the curves for the two were parallel, with a lag of some days between effect on growth rate and effect on susceptibility. Plants that had passed through the susceptible phase and became resistant to mildew, again became susceptible when supplied with more N. Nitrogen-deficient plants continuously resisted infection.

The higher the average growth rate during an experiment the greater was the total amount of infection. Increasing the average growth rate was soon followed by a sharp increase in the amount of infection. When plants of two size groups received the same amount of N the initially smaller plants became more heavily infected than the larger plants.

Many foliage diseases occur only within a limited range of temperature and humidity. Tapke (1951), from an analysis of the weather during epidemics of cereal powdery mildews, did not find a predominant type and consequently concluded that the condition of the host was the important predisposing factor. This can be altered by many factors, of which temperature and nitrogen supply were tested in glasshouse experiments described below.

TREATMENT OF PLANTS

Six grains of the winter wheat variety Red Standard (Squareheads type) were sown in each 7 in. Sherdley pot, which contained 4.1 kg. of a 1:3 mixture by weight of an infertile soil and washed silver sand, both air dried. The sowing dates for the two seasons 1950/51 and 1951/52 were 30 October 1950 and 26 October 1951, respectively. After emergence, the number of seedlings per pot was reduced to five.

Constant amounts of potash and phosphate, 2 g. K_2SO_4 as solution, and 1.5 g. CaH_2PO_4 as solid per pot, were given to all treatments soon after seedling emergence. The amount and date of application of the nitrogenous fertilizer varied. The unit quantity of nitrogenous fertilizer (N) was 0.3 g. $NaNO_3$ plus 0.4 g. $Ca(NO_3)_2 \cdot 4H_2O$ applied as solution. Symbols 0N, 1N, 2N, ... represent treatments where 0, 1, 2, ... units of N were given.

The fungus spread readily from the initial infections which were established in December, naturally in season 1950/51 and artificially in season 1951/52. For inoculation, the upper surface of the second oldest leaf on the main stem of one plant per pot was covered with cardboard except for an area of 0.2 sq.cm., 6.5 cm. from the stem. After this area had been inoculated with dry conidia of *Erysiphe graminis* applied with a camel hair brush the plants were put in a glasshouse. Of the inoculations 99% were successful.

The treatments, for each of which there were at least three replicate pots, were randomly arranged.

METHODS OF ASSESSMENT

(a) *Plant growth.* Successive recordings (in sq.cm.) of the leaf area of one plant per pot were made, by totalling the areas of the blades and sheaths of individual leaves. The area of each leaf blade was estimated by doubling the product of its length and maximum breadth, to allow for both surfaces. This area was corrected by multiplying with a factor giving the relation between the true leaf area (LT), measured with a planimeter, and the area (LB), given by the product of the length and maximum breadth (Lal & Subba Rao, 1951). For Red Standard $LT/LB = 0.78$. The length (l) and the diameter (d) were measured on the exposed part of the leaf sheath whence the area (LS) was given by $LS = l\pi d$.

(b) *Incidence of mildew infection.* The methods used by previous workers were found to be inadequate. As the level of infection was not constant for all the leaves on a stem (see Fig. 5), the method of Smith & Blair (1950), in which the infection on a small random sample of leaves is estimated, introduces large errors. The distribution of pustules on a leaf did not correspond with that used in the 'standard area' diagrams (Grainger, 1947). The method to be described, which is independent of the distribution of pustules on a leaf and allows for leaf to leaf variation in the level of infection, but ignores pustule size, is too laborious to use in the field, where the variation expected would necessitate many large replicate samples.

Infection was recorded on the leaf blades on the main stem of one plant per pot—that used for growth measurements. The numbers of pustules on the upper and lower surfaces and the area of each leaf were recorded, and the number of pustules per unit area, 100 sq.cm., was calculated for each leaf. From the replicates of each treatment the antilogs of the mean logs of both the leaf area and the number of pustules per 100 sq.cm. for each leaf position were taken.

If a_1 , a_2 and a_3 represent the mean leaf areas, p_1 , p_2 and p_3 the mean number of pustules per 100 sq.cm. of three leaves on a main stem, then

$$\frac{a_1 \times p_1}{100} + \frac{a_2 \times p_2}{100} + \frac{a_3 \times p_3}{100} = y,$$

where y = total number of mildew pustules on leaf blades of total area $a_1 + a_2 + a_3$.

To eliminate the plant-size differences between treatments, the infection index is calculated for a total leaf-blade area of 100 sq.cm. and is given by

$$\text{Infection index} = \frac{y \times 100}{a_1 + a_2 + a_3} \text{ pustules per 100 sq.cm.}$$

This treatment of the data does not allow statistical analysis.

EFFECT OF TEMPERATURE

During the winters when the plants were kept in an unheated glasshouse, little spread occurred from the initial infections, although conidia were produced abundantly. Conidia which had caused no infections at a mean temperature of 6.7° C. (44° F.) were collected and examined on a slide coated with glycerine jelly. Germination and the formation of appressoria had not been inhibited.

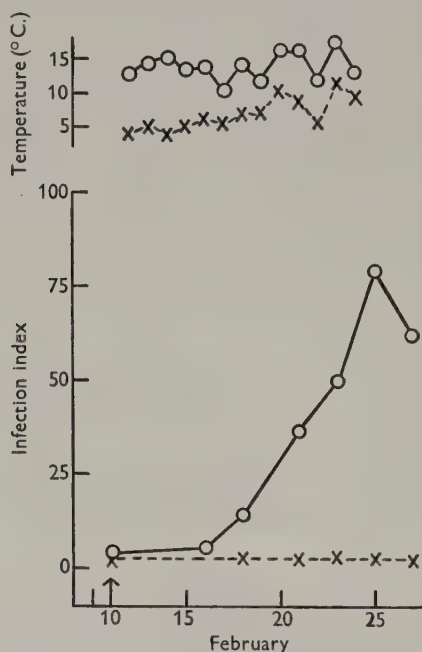


Fig. 1. Effect of temperature on the infection index. ○—○ = mean temperature, 14° C.; ×---× = mean temperature, 6.7° C.

A set of lightly infected plants was divided into two batches; one was left in the unheated glasshouse and the other was placed in a heated glasshouse. The infection-index curves, which join successive recordings of the infection indices, and the daily mean temperatures for the subsequent 16 days are given in Fig. 1. At a mean temperature of 14° C. (57° F.), the infection index appreciably increased after 7 days, whereas no new infections developed at a mean temperature of 6.7° C. At both temperatures the plants increased in size.

Conidia production was measured by enclosing isolated pustules in a chamber similar to that used for localizing insects in virus work (Walrave, 1951). The basal cover-glass on which the conidia were deposited was coated with glycerine jelly. At the end of the test, and to facilitate counting, the heaped deposit of conidia was dispersed while the glycerine jelly was temporarily melted. The number of conidia counted was corrected by dividing by the actual area of pustule to give the number of conidia produced by a pustule of 1 sq.cm. in area. Ten times the number of conidia were produced at 14° C. than at 6·7° C. (Table 1).

TABLE 1. *Effect of temperature on conidia production (conidia produced per sq.cm. of pustule per day)*

Replicates	Mean daily temperature	
	6·7° C. (44° F.)	14° C. (57° F.)
A	$8\cdot5 \times 10^3$	$2\cdot8 \times 10^5$
B	$2\cdot6 \times 10^4$	$3\cdot9 \times 10^5$
C	$1\cdot1 \times 10^4$	$2\cdot9 \times 10^5$

The effect of temperature was further tested using detached leaves taken from plants grown at 7° C. After at least 3 days' pre-inoculation treatment (pre-treatment) at either 7 or 20° C., comparable leaf blades, each with $\frac{1}{2}$ in. of its sheath, were detached and placed singly in test-tubes containing distilled water to a depth of $\frac{1}{2}$ in. These leaves were inoculated evenly on their upper surfaces only, with dry conidia. After inoculation the leaves from each pre-treatment were divided into two batches, one receiving a cold and the other a warm post-inoculation treatment (post-treatment), until infections developed. The results of one of the experiments, which for statistical analysis were transformed into logarithms, are given in Table 2.

TABLE 2. *Effect on infection of pre- and post-inoculation treatments at different temperatures*

	Mean temperature			
	7° C.	7° C.	20° C.	20° C.
Pre-inoculation treatment	7° C.	21° C.	7° C.	21° C.
Post-inoculation treatment				
Mean log of number of pustules per unit area (S.E. $\pm 0\cdot26$)	1·54	2·37	2·45	2·50
Antilog of mean log of number of pustules per unit area	34·7	234·0	282·0	316·0

The number of pustules which developed when both the pre- and post-treatments were at 7° C. was significantly less than when either the pre- or the post-treatments or both were at 20–21° C. The differing effects of pre-treatment at 7 and 20° C. when followed by post-treatment at 7° C. can only be due to a change in the host's

susceptibility. In the post-treatment at 7° C., pustules developed in 10 and 14 days after pre-treatment at 20 and 7° C. respectively. There was no effect of pre-treatment on the rate of pustule development when followed by post-treatment at 21° C.

EFFECT OF NITROGENOUS FERTILIZER

Spinks (1913), Trelease & Trelease (1928) and Grainger (1947) showed that the susceptibility of cereals to *Erysiphe graminis* is increased by applying nitrogenous fertilizers. From two recordings during the life of a winter-wheat crop, Smith & Blair (1950) suggested that the change in susceptibility only occurs when associated with a growth response. The present investigation was made to study in detail the nature of the relation between infection and nitrogen supplied to the host; the nitrogen being always given before the emergence of the three leaves preceding the ears.

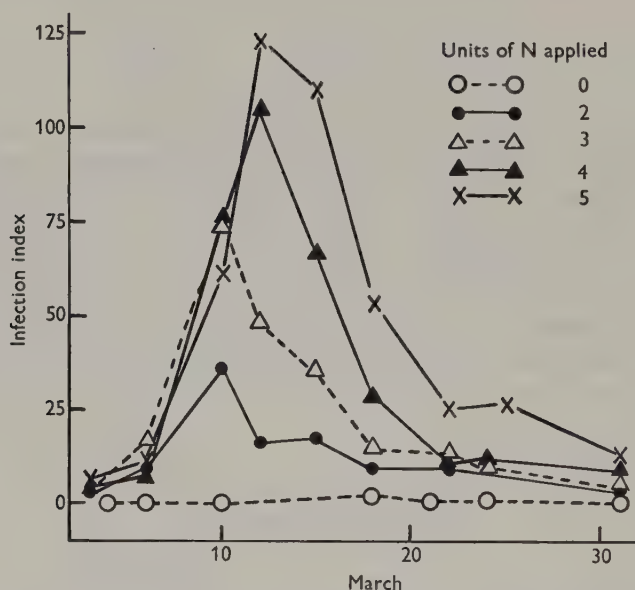


Fig. 2. Infection-index curves of plants given different doses of N.

The infection curves plotted in Fig. 2 are typical for a set of plants given different amounts of N. The data shown are from plants to which the fertilizer was applied at sowing and which were kept in cold conditions unfavourable for infection until 1 March, when they were moved to a glasshouse at 14° C.

As the infection index is calculated for unit area (100 sq.cm.), variations between indices for plants growing in the same environment but given different doses of N can be interpreted as differences in susceptibility. Except for 0N, the infection-index curves for all levels of N are similar in shape but differ in absolute values,

each going through a susceptible phase to a resistant phase. After a month there were no substantial differences between the infection indices. The severity of infection increased with each increase in the level of N. The data for 1N are similar to those for 0N and are therefore not plotted. Both of these treatments produced plants that were nitrogen-deficient before 1 March and resisted infection.

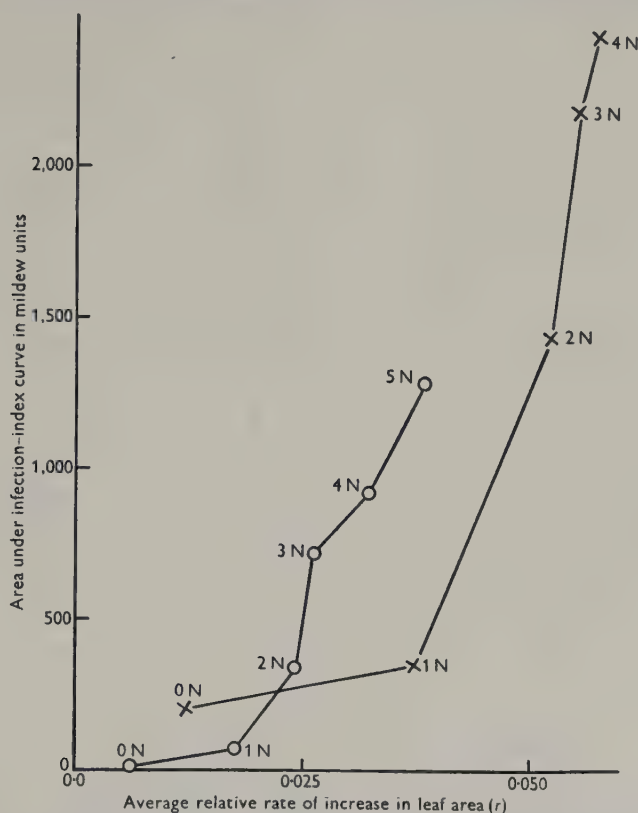


Fig. 3. Relation between the average relative rate of increase in leaf area (r) and the area under the infection-index curves of plants given different doses of N. ○—○ = Exp. 1; ×—× = Exp. 2.

After applying N to plants deficient in this element, but growing in conditions otherwise favourable for the fungus and host, there was a lag period of about 2 weeks before the plants became susceptible.

In Fig. 3 the areas under the infection-index curves are plotted against the average relative rates of increase in leaf area during the period between ' t_1 ' and ' t_2 ' for two experiments (Exps. 1 and 2) in which various amounts of N were tested. ' t_1 ' = date either when plants given N at sowing were moved from 7 to 14° C. (Exp. 1), or when N-deficient plants, already growing in conditions otherwise favourable for infection, were given N (Exp. 2).

' t_2 ' = date when the infection index in the treatment given the highest level of N, had declined to approximately that in oN. The area of the infection-index curve during the period ' t_1 ' to ' t_2 ' is measured in 'mildew units', where a unit is one pustule per 100 sq.cm. for 1 day. The relative rate of increase in leaf area, which for brevity will be signified by ' r ', is given by

$$r = \frac{\log_e a_2 - \log_e a_1}{t_2 - t_1} \quad (\text{Gregory, 1921}),$$

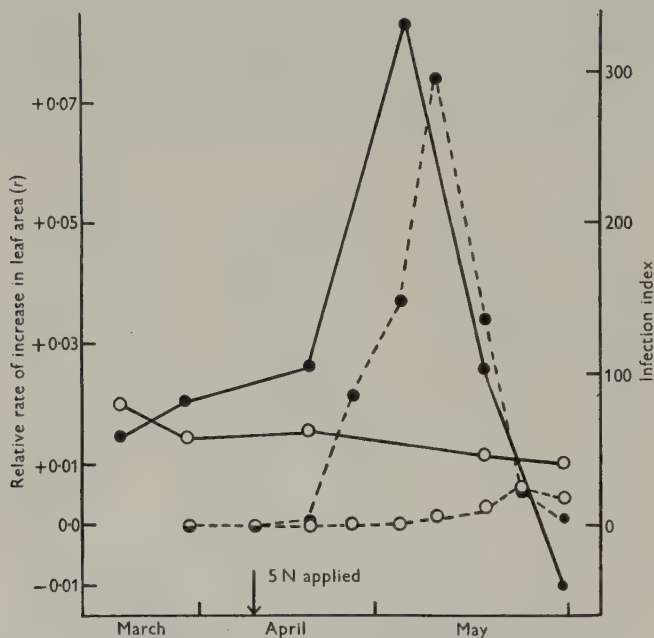


Fig. 4. Relation between successive recordings of the relative rate of increase in leaf area (r) and the infection index. Level of N applied: \circ = oN; \bullet = 5N. \circ — \circ , \bullet — \bullet = curves of ' r '; \circ --- \circ , \bullet --- \bullet = infection-index curves.

where a_1 and a_2 are the leaf areas at t_1 and t_2 respectively. The value of ' r ' in Exp. 1 was corrected for the growth made when the conditions did not allow infection. Here ' t_1 ' was taken as the date when growth differences first appeared. The absolute values of the two curves differ but their shapes are similar. The total area under the infection-index curves increased (a) with increasing amount of N, and (b) as ' r ' increased; the increase with increased ' r ' was not constant. Comparatively large changes in ' r ', at its lowest values in each experiment, were associated with small increases in infection, but at its higher values there were large increases in infection with small changes in ' r '.

Plants given different levels of *N (oN-5N) when sown grew at the same slow rate until plants in treatment oN became nitrogen-deficient in mid-February. Until then the natural infections, which occurred in season 1950/51, were randomly distributed. In season 1951/52 when the plants were artificially infected, there

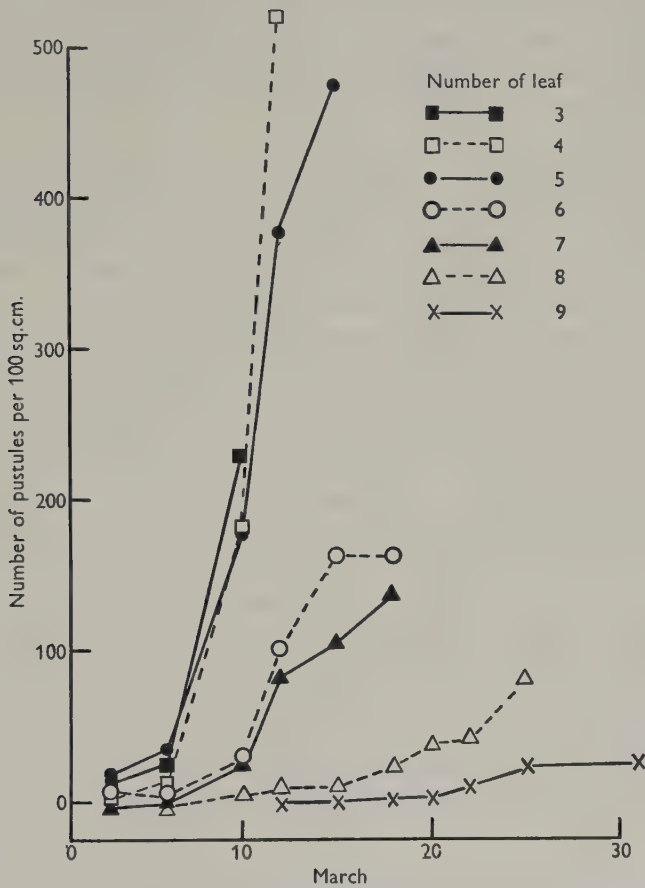


Fig. 5. Infection curves of the individual leaves on the main stem of a plant which passed through a susceptible to a resistant phase.

were no differences between treatments in the percentage of successful inoculations or in the time taken for pustules to develop. Thus plants growing at the same rate were equally susceptible, regardless of the amount of N applied.

In conditions favouring rapid growth, ' r ' did not remain constant. The changes in ' r ' and infection index are plotted in Fig. 4, for two sets of plants, both of which were originally nitrogen-deficient but to one of which 5N was subsequently given. The rate of infection and ' r ' remained approximately constant in treatment oN.

After 5N had been applied 'r' increased to a maximum and then dropped. This curve was paralleled and followed 7 days later by the infection-index curve. The effect of nitrogenous fertilizers in increasing the susceptibility to mildew is therefore only temporary, and the increase is related to the changes of 'r'. This correlation was always obtained, but the time lag between growth response and increase in susceptibility varied. The decline in the value of 'r' is probably associated with the onset of 'internal nitrogen starvation' (Gregory, 1937). The percentage germination and appressoria formation of conidia deposited on resistant and susceptible leaves did not differ. The conidia were collected on slides coated with glycerine jelly.

The infection curves of the individual leaves on the main stem of a plant which passed through this transition are shown in Fig. 5. Here, leaf 3 is older than leaf 4 which is older than leaf 5 and so on. As a heavy inoculum was always present, the slope of the infection curves may be considered a measure of susceptibility—susceptibility increasing with the steepness of the slope. In this example, leaves 3, 4 and 5 were very susceptible, the youngest leaves 8 and 9, which emerged after the start of the susceptible phase, were comparatively resistant, and leaves 6 and 7 were intermediate. These data are from a nitrogen-sufficient plant which was moved into temperature conditions favourable for infection. When N was given to plants deficient in this element, the youngest leaf and the next two or three formed were the most susceptible. The other leaves present when N was applied did not become as susceptible as the youngest but were at least seven times more susceptible than the corresponding leaves on nitrogen-deficient plants.

There was no evidence that leaves of any age were truly resistant and would fail to be infected if conditions of temperature and nitrogen supply were suitably adjusted. Infection per unit area increased with the age of the leaf.

The acquired resistance, adult plant resistance (Graf Marin, 1934), was broken by a further application of nitrogen. In one experiment, after giving 2N to plants in the resistant phase, the area of the ensuing infection curve increased from 152 to 1205 units and the average value of 'r' from -0.006 to $+0.022$.

Effect of plant size

Nitrogen-deficient plants, 5 months old and of two sizes, were randomly arranged among infected plants and given four levels of N. The total leaf areas and the levels of infection of these were 65.2 ± 10.1 sq.cm. and 0.4 pustules/sq.cm. for the smaller plants given no N at sowing and 225.2 ± 22.2 sq.cm. and 1.2 pustules/100 sq.cm. for the larger plants given 1N at sowing. The total area under the infection-index curves for each treatment for the 6 weeks after applying N is given in Fig. 6. At each level of N the initially smaller plants had more than three times as much infection as the initially larger. The smaller plants also had a greater average value of 'r' at the same levels of N.

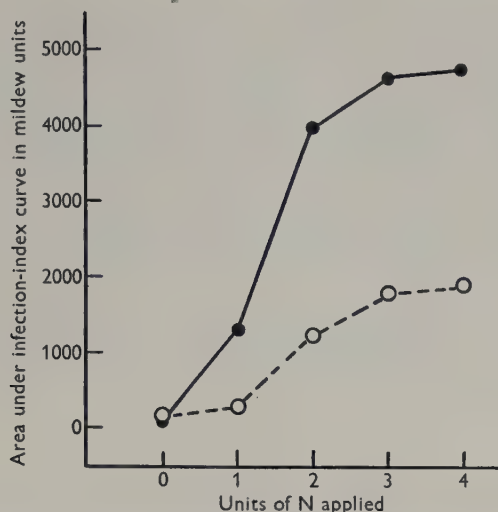


Fig. 6. The effect of initial plant size on the amount of infection subsequent to the application of different doses of N. Initial plant size: ●—● = 65.2 sq.cm.; ○----○ = 225.2 sq.cm.

DISCUSSION

The effects of temperature and nitrogen supply help to explain the occurrence of mildew in field crops.

The mean monthly air temperatures at Rothamsted during the period 1878–1939 for the successive months from November to March inclusive are 42.3, 38.5, 37.6, 38.5 and 41.2° F. respectively. As low temperatures (*c.* 44° F.) are unfavourable for infection, the winter ‘carry-over’ of powdery mildew probably depends on the infections established in the autumn. During the winter plants generally grow slowly, and if in so doing they become nitrogen-deficient, the oldest leaves, which would be those infected in the autumn, are likely to die. Consequently the amount of mildew inoculum persisting to the spring will be greatly reduced.

The amount of mildew infection in the spring on crops of the same cereal often differs greatly within a limited area. These differences in susceptibility may be varietal or due to different applications of nitrogenous fertilizer. The greater the amount of nitrogen applied, the higher is the average rate of increase in leaf area and the greater the total infection, although increases depend on the size of the plant when the conditions start to favour growth. The smaller the plants at this stage, the higher their subsequent relative rate of increase in leaf area and the greater their susceptibility is likely to be. Smith & Blair considered that applying nitrogenous fertilizer increases infection because the increased plant growth changed humidity in favour of the fungus. Cherewick (1944) and others have shown that the percentage germination of conidia on glass slides increases as the relative humidity

approaches 100%. In the experiments described above the replicate pots of each treatment were randomized, so that humidity differences were unlikely to occur except near to the leaf surface. The conidia deposited on susceptible and resistant leaves did not differ significantly in the extent to which they germinated and produced appressoria. This does not support the idea that increased susceptibility produced by nitrogen depends on a change in the external environment of the plant, but suggests that it depends on physiological changes within the leaves.

An early high level of infection in the spring in field crops is often not maintained later. The crop is said to 'grow out' of the disease. This phenomenon probably reflects internal changes in the plant and not changes in the climate within the crop; it is probably attributable to a depletion of the nitrogenous compounds which are necessary to maintain a high rate of growth of both the host plant and the fungus.

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STUDIES ON A CANKER DISEASE OF CYPRESSES IN EAST AFRICA, CAUSED BY *MONOCHAETIA UNICORNIS* (COOKE & ELLIS) SACC.

I. OBSERVATIONS ON THE PATHOLOGY, SPREAD AND POSSIBLE ORIGINS OF THE DISEASE

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(With Plate, 2 Maps and 6 Text-figures)

A canker disease of cypresses, caused by *Monochaetia unicornis*, was first observed in Kenya in 1942. Outbreaks appear to have followed a spread from south-east to north-west, the direction of the south-east monsoon, and the disease has now been recorded in nearly all the cypress-growing areas of Kenya, Tanganyika and Uganda, in Southern Rhodesia and the Union of South Africa. The oldest known infections occurred in 1937.

The fungus grows in the bark and sapwood, forming cankers which spread rapidly up and down the stem or branch, more slowly round, frequently causing girdling. Cankered trees produce large amounts of resin and a periderm may be initiated in advance of the fungus. Infections occur most commonly at main branch crotches, but cankers also start at the tree collar, on the stem and at minor branch crotches.

The fungus exists in nature in three strains having differing levels of virulence, growth rate in culture, size of acervuli and conidia, and retention of viability in conidia. The distribution of one strain appears to be limited by ecological conditions.

Acervuli ripen and conidia are liberated from 3 to 8 weeks after the start of the rains. Infections start during the rains and misty weather, the slimy spores being dispersed for short distances only. Conidia may retain their viability for up to 28 months.

The local spread of the disease in Kenya has been traced and differing levels of virulence east and west of the Great Rift valley, associated with differences of climate, have been observed.

Trees 2 years old are the most susceptible and older trees are only infected in the rapidly growing parts. *Cupressus macrocarpa* is more susceptible than *C. lusitanica*, the latter species being heavily infected only where it is growing close to diseased *C. macrocarpa*.

Occlusion of cankers occurs in both species but is more effective in halting the spread of the fungus in *C. lusitanica*.

It is suggested that the disease originated from a single focus and may represent the mutation of a strain of *Monochaetia unicornis* parasitic on cypresses from a strain only parasitic on an indigenous host, *Juniperus procera*. The fungus may, however, have been imported from abroad.

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INTRODUCTION

A canker disease of *Cupressus macrocarpa* in East Africa was first observed by Wimbush in 1942 (Wimbush, 1944). Nattrass (1945) carried out a pathogenic study and identified the causative fungus as *Monochaetia unicornis* (Cooke & Ellis) Sacc. Serious losses in plantations followed the first outbreak of the disease, and investigations were continued at the Scott Laboratories of the Department of Agriculture, Nairobi (Nattrass, 1946, 1948, 1949, 1950; Nattrass & Ciccarone, 1947). Early in 1949 the writer was appointed to carry out a full-scale investigation.

The present paper gives an account of field studies of the disease. Further papers will be concerned with the biology of the causative fungus and the resistance and susceptibility of species of *Cupressus* and allied genera.

The planting of cypresses in East Africa

Cypresses were first planted in East Africa using seed of *Cupressus* sp. imported from Guatemala in 1909-10 (Wimbush, 1945). Experimental plots of various *Cupressus* species were started in Tanganyika by the Germans as early as 1908, and similar plots were planted at Entebbe in Uganda in the 1920's. Extensive commercial planting in Kenya commenced in 1926.

Plantations have now been established over all the Highland areas of East Africa where there is an average annual rainfall of more than 30 in., and ornamental cypresses are commonly planted in areas with less rainfall. *C. macrocarpa* and *C. lusitanica* are the most extensively planted species.

Seedlings are raised in forest nurseries and are planted out in the first year of growth at 8 × 8 ft. The final crop, on a 35-year rotation, is 80-100 trees per acre.

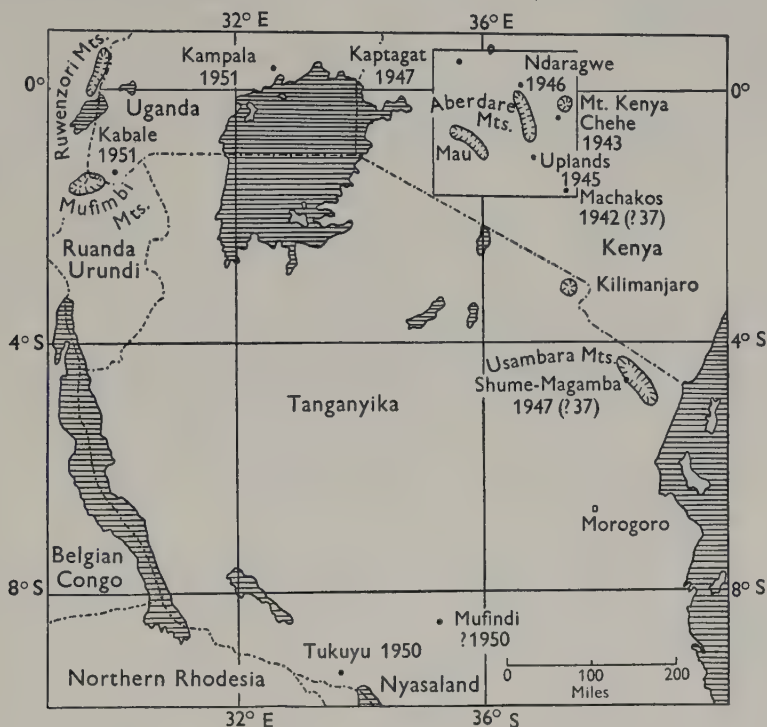
OUTBREAKS OF THE DISEASE

The disease was first observed by Wimbush on *C. macrocarpa* growing on the Iveti Hills above Machakos, 40 miles south-east of Nairobi (Map 1). Recent examination of the butt of an infected tree which had recovered from the disease, showed that infection took place about 1936-7. These cypresses are 50 miles from the nearest plantations of the same species at Uplands on the southern end of the Aberdare Mountains. Examination of further infected trees from Shume Magamba in the West Usambara Mountains, 230 miles to the south-south-east, where the disease was first reported by Wallace (1949), showed that the disease must have first appeared there at about the same time as at Machakos.

The disease next appeared at Chehe on the southern slopes of Mt Kenya (75 miles north of Machakos) in August 1943, and was active at Uplands in July 1945. During 1946 the disease was also observed at Ndaragwe at the northern end of the Aberdare Mountains. The first record west of the Rift Valley was at Kaptagat in July 1947, although it was probably present at Elgeyo during 1946 (confirmed in 1950) (Map 2).

The fungus was isolated from an infected *C. lusitanica* tree at Tukuyu in the Southern Highlands of Tanganyika in November 1950. No cankers over 3 years old were found, although Wallace (1950) had found two suspected cankers in 1948.

In Uganda, a suspected canker was found on *C. sempervirens* var. *pyramidalis* at Kampala in March 1951. The disease was identified on *C. macrocarpa* in the same month at Kabale near the Belgian Congo border. No cankers more than a year old were found.



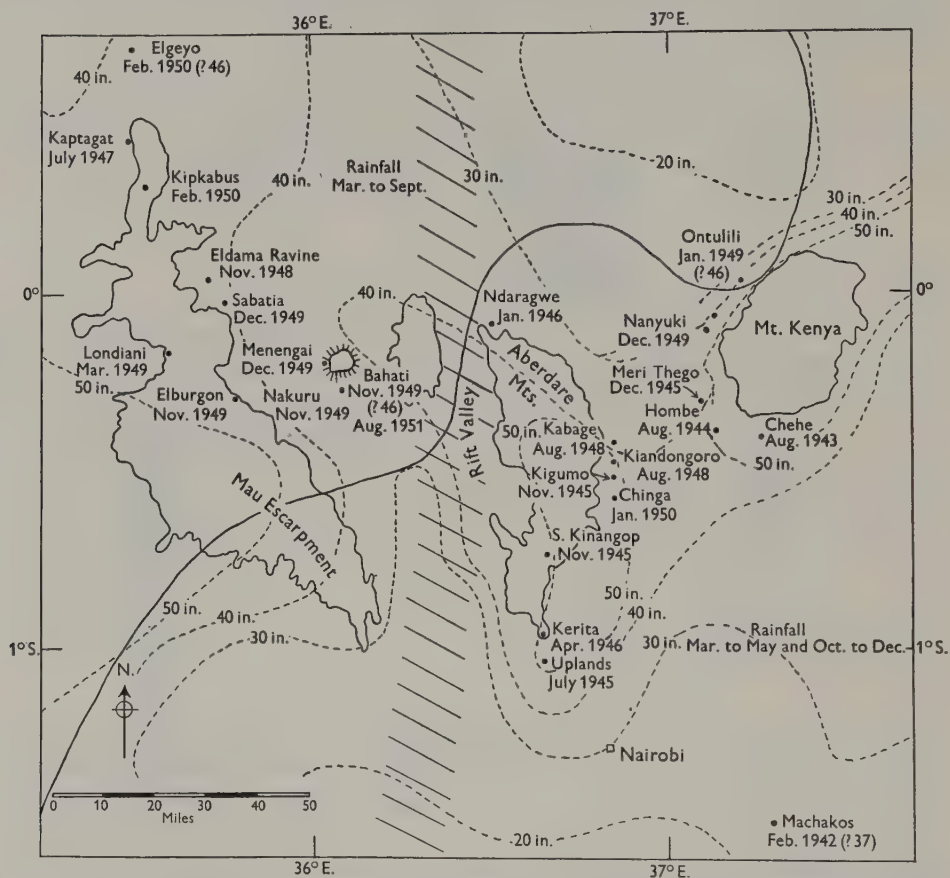
Map 1. The spread of canker disease in East Africa. Inset is the area covered by Map 2.

Dr Natrass found material of *Monochaetia unicornis* on a herbarium specimen of *Cupressus lusitanica* in the Department of Agriculture, Division of Botany and Plant Pathology, Pretoria, in 1949, but the disease has not been reported from plantations in the Union. Griffith (1951) reported the disease on *C. macrocarpa* at Stableford in Southern Rhodesia.

All the early infections were of *C. macrocarpa*, but in July 1947 the disease was reported for the first time on *C. lusitanica* at Chehe, and it has since been reported on this species in most areas where the disease is active. *C. sempervirens*, *C. arizonica*, *C. benthami* and *C. forbesii* have also been found naturally infected.

In September 1951, the fungus was collected from a branch die-back of a naturally regenerated tree of the East African pencil cedar, *Juniperus procera*, at

Nanyuki. There was no obvious canker lesion or resin flow, and the conidia were found to be larger and darker in colour than those of cypress collections. In October, a further collection was made on *J. procera* in a plantation at Kabage. In this instance there was a typical though limited canker lesion and the conidia did not differ from those of the cypress collections.



Map 2. The spread of canker disease in Kenya. Continuous black lines enclose land over 2500 m. (8200 ft.). The continuous black line running from top right to bottom left separates the two rainfall zones, the broken lines being isohyets.

SYMPTOMS OF THE DISEASE

The first symptom of the disease is normally a flow of resin (Pl. 3, fig. 1), followed by a canker, the fungus mycelium ramifying in the bark and sapwood which become infiltrated with resin. The canker extends rapidly up the stem or branch, more slowly downwards and round, but eventually girdles the stem or branch which then dies back above the infection. (Pl. 3, fig. 2).

Three to five weeks after infection, black, crater-like acervuli erupt through the surface of the bark close to the point of infection. Further crops of acervuli may be produced as the canker extends.

Mode and foci of infection

Infection is usually by conidia but occasionally by ascospores (Natrass, 1950). There is a tendency for the bark tissues to show surface cracks under the rapid growing conditions of East Africa, and this is particularly noticeable at the branch crotch, where the secondary thickening of the stem impinges against that of the branch to form a 'cushion' or 'ruffle' of distorted bark which often shows minute cracks. Conidia could readily lodge in these cracks and presumably give rise to infections.

TABLE I. *Foci of canker infections on Cupressus macrocarpa and C. lusitanica during 1949-51*

Species	Year of planting	Number of trees	Number of trees infected	% trees infected	Crotch 1	Crotch 2	Stem 1	Stem 2	Branch 1	Branch 2	Collar 1	Collar 2	Stump cankers	2 types	3 types	4 types
<i>C. mac.</i>	1945-6	441	322	73.0	68	90	92	123	39	27	6	1	40	87	37	1
<i>C. lus.</i>	1944-6	685	103	15.0	20	3	48	20	10	1	5	0	6	8	1	0
<i>C. lus.</i>	1947	323	53	16.4	7	5	26	4	7	1	6	0	3	6	0	0
{ <i>C. mac.</i>	1948	162	151	93.2	44	97	6	1	8	7	1	0	1	14	0	0
{ <i>C. lus.</i>	1948	167	86	51.5	39	34	7	0	4	1	5	0	3	7	0	0

Crotch 1 signifies one canker at a branch crotch; crotch 2, two or more; stem 1 signifies one canker on the stem; stem 2, two or more; branch 1 signifies one canker on a branch other than at the crotch; branch 2, two or more, not necessarily on the same branch; collar 1 signifies one canker at the collar; collar 2, two or more; stump cankers signify trees killed by canker disease at first recording and felled; 2, 3 and 4 types signify numbers of trees with cankers in 2, 3 or 4 different foci (e.g. 1 branch crotch canker and 1 stem canker). The numbers for 2, 3 and 4 types are also included under separate foci headings.

The bracketed, 1948 planted, *C. macrocarpa* and *C. lusitanica* were in a 'strip-by-strip' plantation. All the trees planted between 1944 and 1947 had been pruned once, after 3 years' growth, to half-height.

Table I analyses the foci of infection in sample plots of infected *Cupressus macrocarpa* and *C. lusitanica* trees in the plantation areas of Mt Kenya and the Aberdares. In trees under 3 years old, infections were practically confined to the branch crotch and collar. In trees 3-7 years old, there were more stem cankers, which had frequently originated on pruning scars or wounds or were centred at the crotches of very small branches. Infections of the stem also originated in wounds caused by game, particularly buffalo and bushbuck. No specific instances of infection following insect damage were observed.

Host reaction

Tissues of the bark, cortex, cambium and newly formed wood are killed by the fungus and older wood may be invaded. Suberization followed by periderm formation may provide a barrier to further mycelial advance, and the abundant production of resin may constitute a further check. Periderm barriers are penetrated by hyphae, especially in weather favouring the rapid extension of the canker, and it is common to find a succession of such barriers which have been crossed by the fungus.

The cambium is normally killed at the upper and lower ends of the canker, but at the sides it divides rapidly to form new bark and wood which occlude the lesion. This activity of the cambium often gives the stem a winged appearance (Pl. 3, figs. 3, 4), and the weakening of the stem produced by a canker followed by the reaction of the host tissues sometimes results in a serious twisting (Pl. 3, fig. 5).

ISOLATION OF *MONOCHAETIA UNICORNIS*

Early in the investigation it was apparent that *Monochaetia unicornis* existed in a number of 'strains' or biotypes which could be distinguished in culture. Nattrass & Ciccarone (1947) reported both pathogenic and saprophytic strains, and Nattrass (1949) reported a strain forming albino acervuli with completely hyaline conidia. In the present investigation three distinct strains, A, B and D, have been recognized in nature and a further strain, C, which is non-pathogenic, sterile and slow-growing, has arisen in culture. Strain A is virulent, producing extensive cankers, fast-growing in culture but forming few acervuli. Strain B is less virulent, slow-growing in culture but forming many acervuli which are larger than those of strain A and the conidia generally have longer setae and are darker. Strain D has only been isolated from *Juniperus procera*, and it is non-pathogenic on all the *Cupressus* species tested. It grows more slowly than strain B in culture and forms a few acervuli similar in size to strain A, although the conidia are larger and darker than those of both strains A and B.

Forty-six collections of the fungus have been established, and a further six obtained through the courtesy of Dr Nattrass have been included in various studies. Of these collections, thirty-nine correspond to strain A, ten to strain B, two to strain C and one to strain D.

The perfect stage of the fungus

Nattrass (1950) placed the perfect stage in the Phoepphragmiae of the Sphaeriales, but the species is so far undescribed.

Table 2 shows that perithecia are produced quite commonly in nature, and field observations show that they are formed more regularly on *Cupressus macrocarpa* than on *C. lusitanica*. They have been obtained both from wet (e.g. Kerita) and dry (e.g. Menengai) sites.

The period of time after infection when perithecia are first formed has not been determined accurately, but field observations indicate at least 6 months. One collection established from a conidium produced perithecia after 8 months in culture on a sterilized *C. lusitanica* twig.

TABLE 2. *Occurrence of perithecia in natural and inoculated cankers*

Origin of canker	No. of trees	No. of trees with perithecia
Natural (over 6 months old) on <i>C. macrocarpa</i> and <i>C. lusitanica</i>	72	10
Inoculated 3-year-old <i>C. lusitanica</i> : 12 months after inoculation	150	6 (3 RM9; 2 RM4; 1 RM8)
Inoculated 3-year-old <i>C. lusitanica</i> : 9 months after inoculation	95	5 (1 NM1313; 1 RM33; 3 RM31)

The numbers in brackets are of the collections of the fungus used in the original inoculations.

DISSEMINATION AND VIABILITY OF CONIDIA

The conidia of *Monochaetia unicornis* are extruded from the acervuli in slimy masses during the rains or misty weather. Spore-catching experiments using Petri dishes and slides were carried out during July and August 1950 and 1951, at Kerita, where the incidence of ground mists is high. Table 3 gives details of the results.

TABLE 3. *Trapping of airborne conidia of Monochaetia unicornis 20-21 July 1951. Weather overcast following light rain; wind Beaufort 0-1. Exposed surface 5 cm.² on glass slides.*

Position of glass slide	Exposure time in hr.	Total no. conidia trapped	No. conidia per cm. ² per hr.
5 cm. below ripe acervuli	5½	1623	59.0
20 cm. below ripe acervuli	5½	2131	77.5
30 cm. below ripe acervuli	5½	189	6.9
40 cm. leeward side of ripe acervuli and 90 cm. lower	5	1	> 1
90 cm. leeward side of ripe acervuli and 90 cm. lower	5	2	> 1
140 cm. leeward side of ripe acervuli and 90 cm. lower	5	2	> 1
240 cm. leeward side of ripe acervuli and 90 cm. lower	5	0	0
25 m. leeward side of infected plantation (average tree 35 m. high)	5	1	> 1
100 m. leeward side of infected plantation (average tree 35 m. high)	5	1	> 1

During the rains and mists, water running down the stem will dislodge spores from acervuli. The results from the trapping experiments show that a large proportion of conidia do not travel very far from the tree but occasional spores may be carried up to 100 m.

Table 4 gives the results of viability tests carried out with collections of the fungus from various sources. Conidia from strain A collections generally retained

TABLE 4. *Effect of storage on viability of conidia of Monochaetia unicornis*

Collection no.	Host	Strain	Source	Storage period in months	% germination
RM ₄	<i>C. lusitanica</i>	A	Natural canker	12	0
RM ₉	<i>C. macrocarpa</i>	A	Twig culture	8	13.0
			Twig culture	15½	0.5
			Twig culture	20½	0
RM ₁₀	<i>C. macrocarpa</i>	A	Twig culture	14	81.5
			Twig culture	19½	62.5
			Twig culture	27	48.7
RM ₁₅	<i>C. macrocarpa</i>	A	Natural canker	12	48.0
			Natural canker	19½	0
RM ₁₇	<i>C. lusitanica</i>	A	Twig culture	9	22.2
			Twig culture	14	23.8
			Twig culture	21½	0
RM ₂₇	<i>C. macrocarpa</i>	A	Twig culture	15½	20.0
			Twig culture	20½	16.8
			Twig culture	28	1.0
RM ₃₄	<i>C. lusitanica</i>	A	Twig culture	6	15.0
				11½	0
RM ₄₀	<i>C. macrocarpa</i>	A	Twig culture	5	83.3
			Twig culture	7	30.0
RM ₄₅	<i>Juniperus procera</i>	A	Twig culture	5	90.5
			Twig culture	7	0
NM ₂₄	<i>C. macrocarpa</i>	A	P.D.A.	12	0
NM ₁₃₁₃	<i>C. macrocarpa</i>	A	P.D.A.	12	0
			Twig culture	8	17.0
			Twig culture	15½	17.7
			Twig culture	20½	3.1
			Twig culture	28	0
RM ₁	<i>C. macrocarpa</i>	B	Natural canker	14	6.5
			Natural canker	22	0
RM ₂₃	<i>C. forbesii</i>	B	P.D.A.	8	0
			Twig culture	9	0
RM ₃₅	<i>C. macrocarpa</i>	B	Twig culture	6	0
RM ₄₁	<i>C. macrocarpa</i>	B	Twig culture	5	49.5
			Twig culture	7	22.9
NM ₂₃₂	<i>C. macrocarpa</i>	B	Twig culture	9	0
RM ₄₄	<i>Juniperus procera</i>	D	Twig culture	5	4.6
			Twig culture	7	0

All collections gave over 90% germination when first tested.

their viability for longer periods than those of strain B collections. The only test so far carried out with strain D suggests a shorter retention of viability in this strain. Under natural conditions exposure to rain and sunlight would reduce the viability more rapidly, as Dimock has shown for *Coryneum* canker in California (reported by Wagener, 1948).

Dissemination and viability of ascospores

Under moist conditions ascospores are extruded from the beak of the perithecium in a slimy mass. Ascospores from perithecia on six natural cankers left in the open outside the laboratory gave over 50% germination after 6 months' exposure.

Ascospores from one natural canker kept in the laboratory for 1 month gave 95% germination.

Conidia and ascospores collected from the same tree gave 82.0 and 93.4% germination respectively on collection and 40.0 and 73.5% germination after 8 months' storage in the laboratory.

There is no evidence so far as to how the long-distance dissemination of the disease occurs nor whether conidia or ascospores, or both, are involved.

THE SPREAD OF THE DISEASE

The main outbreaks of the disease in East Africa (Map 1) appear to follow a general spread from south-east to north-west. Such a spread might be attributed to wind dispersal of spores by the south-east monsoon. It is difficult to interpret the outbreak at Tukuyu on such a basis unless the disease spread from plantation areas farther south. Between Tukuyu and the Usambara Mountains there are cypress plantations at Mufindi where one suspected canker was found in November 1950 on *Cupressus macrocarpa*, but no further infections have been reported from the area. There are also plantations in the Uluguru Mountains near Morogoro, but a careful inspection in 1950 did not reveal the presence of the disease. The strain of *Monochaetia unicornis* present at Tukuyu does not appear to differ from collections of strain A made in Kenya.

Within Kenya, the spread of the disease can be followed closely (Map 2). Long-distance spread between plantation areas must have occurred from Machakos to Chehe and Uplands and to Elgeyo (or Kaptagat), and within plantation areas the local spread can be traced. Thus strain A, which was actively producing the disease at Chehe in August 1943, had spread to Hombe, 10 miles west, by August 1944 and 6 miles farther north-west to Meri Thego by December 1945. At the same time it had spread to Kigumo on the western slopes of the Aberdares. In the same area following the first infection of *C. lusitanica* at Chehe in July 1947 the disease was recorded in this species on the Aberdares in August 1948 at Kabage and Kiandongoro and farther south at Chinga in January 1950.

A similar local spread occurred from Uplands to Kerita and South Kinangop on the Southern and Western Aberdares.

Local spread west of the Rift Valley is more difficult to interpret. It is probable that the disease was first active at Elgeyo in 1946 and Kaptagat in 1947. It did not spread to Kipkabus, which is 10 miles to the south, until late in 1949 (confirmed March 1950), although there is only a very small gap between the cypress plantations of the two areas. In this instance, however, the spread was against the direction of the prevailing wind. The outbreaks at Eldama Ravine and Sabatia in 1948 and 1949 may have been initiated by spores dispersed from Kaptagat or from the Aberdares.

The spread of strain B cannot be so clearly defined. It was probably present at Ontulili in 1946 and has since appeared in both *C. macrocarpa* and *C. lusitanica* in

other plantations in the same area and farther south at Nanyuki. Strain B was active at Ndaragwe in 1946, although the identity of the strain was not established until 1951, and has also been isolated from *C. macrocarpa* at Bahati, *C. arizonica* near Nakuru, *C. macrocarpa* on Menengai, *C. forbesii* in a trial plot at Elburgon and *C. macrocarpa* at Londiani, all in November and December 1949.

In August 1951, strain A was found in a number of plantations at Bahati, although only strain B was present when the area was first visited in December 1949. In one plantation of 6-year-old *C. macrocarpa* both strains were present; in ten trees examined eight had been infected with strain A and two with strain B, and the two strains were never present together on the same tree either in the perfect or imperfect stage. It appears that strain A may be replacing strain B on the wetter sites at Bahati, but further observation is required. In every other instance strain B has been isolated from plantations in low rainfall areas.

The effect of climate on the spread and development of the disease

Table 5 shows the average annual rainfall for cypress-growing areas where the disease has been recorded together with the hosts and strains of the fungus isolated.

The average annual rainfall for cypress planting in East Africa varies between 30 and 100 in., and the number of rain days is generally over 100 with few exceptions. The distribution of the rainfall (Map 2) differs east and west of the Rift Valley. East of the Rift, the rainfall occurs in two seasons, March to May with a maximum in April, and October to December with a maximum in November. West of the Rift there is a single season with maxima in May and August. Rainfall is similar in the Uganda cypress areas with the exception of those near Lake Victoria, where there is no definite rainfall season but maxima in April and December. In Tanganyika there is one season from December to May with a maximum in March, and in the south there may be also an early maximum in December. The long rains east of the Rift in Kenya are followed by a period of sunny weather in June, and then overcast conditions accompanied by heavy ground mists which may last throughout the day during July, August and occasionally September. Under these conditions the relative humidity remains high, so that the only really dry season is from January, when the short rains end, until March when the long rains start. Similar misty conditions are experienced, though they last for shorter periods, at Kabale in Uganda, and the Southern Highlands province of Tanganyika.

The disease develops and spreads rapidly east of the Rift where ground mists persist in plantations, and ripe acervuli are formed and new infections take place from April until September and again from November to January. Text-fig. 1 shows the effect that ground mists have in the maintenance of a high relative humidity. During 1950 continuous hygrograph records were taken at Chehe and Hombe (fig. 1A). The former site provided most favourable conditions for the rapid spread of the disease, so that all *C. macrocarpa* plantations had to be clear-felled within 4 years of the first appearance of the disease. At Hombe the disease has not been so

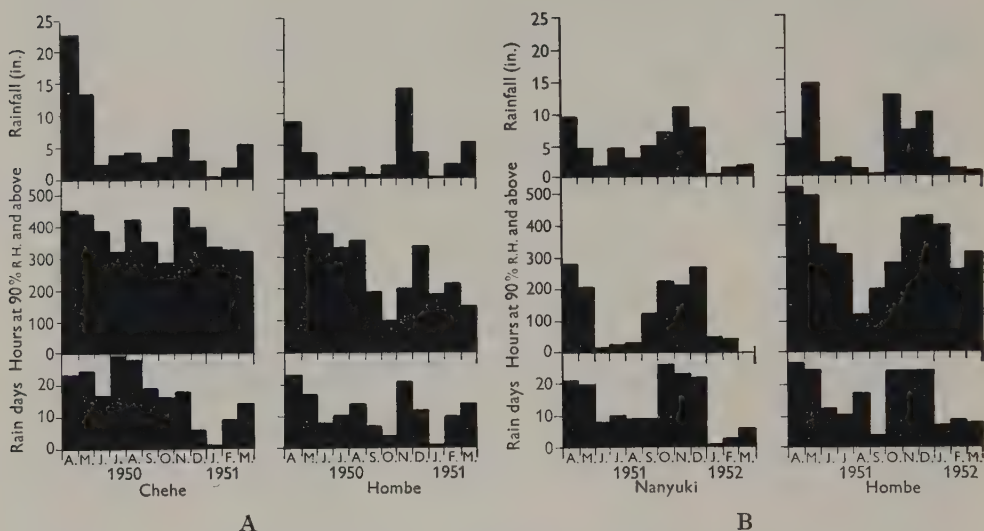
TABLE 5. Annual rainfall and rain days for cypress-growing areas in East Africa with the hosts of *Monochaetia unicornis* and the strains isolated.

Plantation area	Mean annual rainfall (in.)	No. rain days 1950	Hosts of <i>M. unicornis</i>	Strain of <i>M. unicornis</i>
Kenya				
Machakos	49.92	63	<i>C. macrocarpa</i>	A
Chehe	(77.32)	214	<i>C. macrocarpa</i>	A
			<i>C. lusitanica</i>	
Hombe	(56.53)	149	<i>C. macrocarpa</i>	A
			<i>C. lusitanica</i>	
Meri-Thego	34.02	181	<i>C. macrocarpa</i>	A
Nanyuki	34.54	121	<i>C. macrocarpa</i>	B, D
			<i>C. lusitanica</i>	
			<i>Juniperus procera</i>	
Ontulili	29.56	91	<i>C. macrocarpa</i>	B
			<i>C. lusitanica</i>	
Kabage	44.01	157	<i>C. macrocarpa</i>	A
			<i>C. lusitanica</i>	
			<i>Juniperus procera</i>	
Kiandongo	62.74	157	<i>C. macrocarpa</i>	A
			<i>C. lusitanica</i>	
Kigumo	(no gauge)		<i>C. macrocarpa</i>	A
			<i>C. lusitanica</i>	
Chinga	(58.45)	67	<i>C. macrocarpa</i>	A
			<i>C. lusitanica</i>	
S. Kinangop	(38.18)	165	<i>C. macrocarpa</i>	A
			<i>C. lusitanica</i>	
Kerita	53.23	158	<i>C. macrocarpa</i>	A
			<i>C. lusitanica</i>	
Uplands	55.11	159	<i>C. macrocarpa</i>	A
Ndaragwe	31.06	116	<i>C. macrocarpa</i>	B
Bahati	47.84	118	<i>C. macrocarpa</i>	A, B
Nakuru	34.14	—	<i>C. arizonica</i>	B
Menengai	39.49	161	<i>C. macrocarpa</i>	B
Elburgon	41.32	121	<i>C. forbesii</i>	B
Londiani	43.31	149	<i>C. macrocarpa</i>	B
Sabatia	40.96	130	<i>C. macrocarpa</i>	A
Eldama Ravine	43.48	120	<i>C. macrocarpa</i>	?
			<i>C. lusitanica</i>	
Kipkabus	46.14	141	<i>C. macrocarpa</i>	A
Kaptagat	47.29	101	<i>C. macrocarpa</i>	A
Elgeyo	48.25	105	<i>C. macrocarpa</i>	A
Tanganyika:				
Shume-Magamba	43.06	205	<i>C. macrocarpa</i>	A
			<i>C. lusitanica</i>	
Mufindi	80.89	157	<i>C. macrocarpa</i>	?
Tukuyu	99.21	158	<i>C. lusitanica</i>	A
Uganda:				
Kampala	47.58	76	<i>C. sempervirens</i>	?
Kabale	38.69	130	<i>C. macrocarpa</i>	B

Figures in brackets are inches rainfall for 1950 for those stations where the records are for 2 or 3 years only. Rainfall figures for 1950 were near to the mean in most areas.

The identity of the strain or strains in each area was not established until after the writer started his investigation in May 1949.

serious and *C. macrocarpa* plantations were not clear-felled until 6 or 7 years after the first infections. During 1951-2 conditions were compared at Hombe and Nanyuki (Text-fig. 1 B). In the latter area, where ground mists are infrequent, only strains B and D have been recorded and the disease has never been serious.



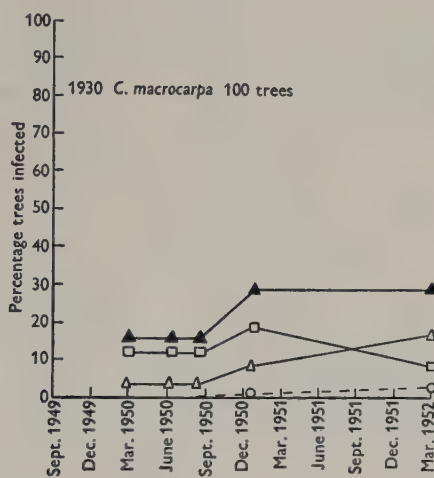
Text-fig. 1. A, monthly rainfall, relative humidity (hours at 90 % R.H. and above) for April 1950 to March 1951 at Chehe and Hombe. B, same for April 1951 to March 1952 at Nanyuki and Hombe.

The difference between areas with and without ground mists is seen also in the spread of the disease through a plantation. West of the Rift, with a long dry period, the progress of the disease is almost halted at the end of the rains, and spread within a plantation appears more typical of a disease caused by a root-invading fungus with a slowly advancing margin, infections occurring most frequently on trees adjacent to those already infected. East of the Rift the spread of the disease through plantation areas has been extremely rapid and without a clearly defined margin.

THE PROGRESS OF THE DISEASE IN PLANTATIONS

Sample plots, normally two to four each of 100 trees planted, have been recorded for various age classes of *C. macrocarpa* and *C. lusitanica* in a number of areas on Mt Kenya and the Aberdare Mountains. No sample plots have been recorded west of the Rift, as it has been the policy of the Kenya Forest Department to fell all trees immediately they are infected in that region. Similarly, with the exception of the 1948 planting at South Kinangop it has not been possible to plot *C. macrocarpa* and *C. lusitanica* growing together on the same site, since the planting of *C. macrocarpa* was abandoned, for silvicultural reasons, on Mt Kenya and the

East Aberdares in 1939, although it was the main species planted on the South and West Aberdares. Recordings were usually made three times during the year: (1) after the long rains, (2) in the dry season between the short and long rains, and (3) during the long rains. Recordings covered the foci of infection of all cankers, trees killed by the disease and trees showing recovery. A canker was described as 'recovered' when there was no fresh flow of resin from the lesion and when the host tissues were obviously occluding the canker. Trees described as 'recovered' during the dry season were often recorded as having active cankers during the following rains, when there was renewed fungal growth.



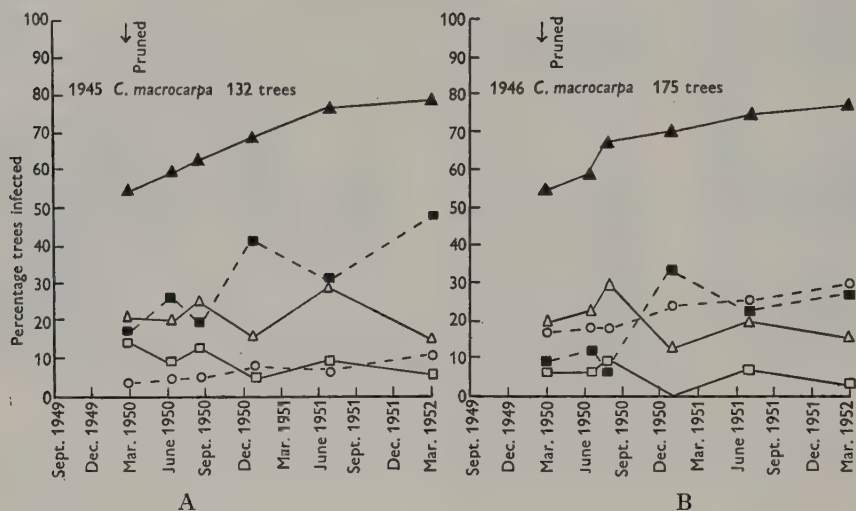
Text-fig. 2. The progress of canker disease in sample plots, 1930 planting. □—□, trees with one active canker (early); △—△, trees with two or more active cankers (late); ○—○, trees killed (dead); ■—■, trees showing recovery; ▲—▲, total number of infected trees. (Legend also applies to Text-figs. 3 and 4).

Effect of age of host

Some of the oldest *C. macrocarpa* have been seriously attacked by the disease. New infections are invariably confined to the actively growing parts of the tree in the unpruned crown. Text-fig. 2 shows the development of the disease in 1930-planted *C. macrocarpa*. The trees were nearly 100 ft. high and accurate recording of cankers was not possible, only 'early', 'late' and 'dead' trees being marked. New infections appeared between August 1950 and January 1951 but were probably initiated in the heavy long rains of 1950. No further infections occurred during 1951, although the rains were good, and in the March 1952 recording three trees had been killed and others were in an advanced stage of the disease.

In 1940-planted *C. lusitanica* on Mt Kenya, no new infections have been recorded since September 1949, and all existing infections have been completely occluded. 1944-planted *C. lusitanica* in the same area showed no new infections after March 1950.

In 1945-6-planted trees the initial infection figures were high (54.5 and 54.8% in *C. macrocarpa* (Text-fig. 3) and 8 to 16% in *C. lusitanica*). In *C. macrocarpa* the number of new infections continued to rise at a steady rate, but showed signs of falling off after June 1951 when the total number of infected trees were 78.6 and 76.5%. The falling off in the number of new infections may have been due to the fact that the remaining trees were resistant, but this was not borne out by the recordings from younger plantations.



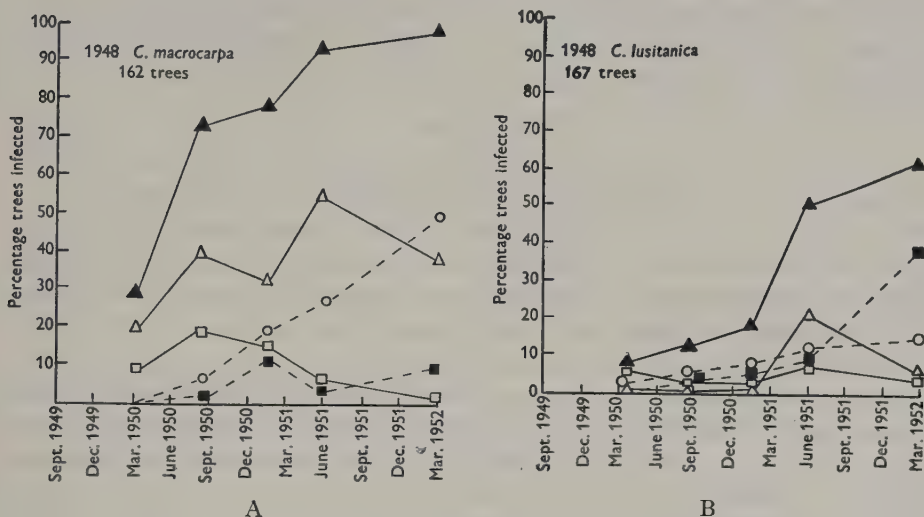
Text-fig. 3. The progress of canker disease in sample plots, 1945-6 planting.

In 1947 planting of *C. lusitanica* infections had risen to 22% in one plantation area on the Aberdares by March 1952, although in a plantation on Mt Kenya only 10% of the trees were infected. The difference cannot have been due to a difference in the amount of inoculum available, since this was certainly greater on the Mt Kenya site but may in some way have been connected with the later appearance of the disease on the Aberdares.

In 1948 planting, it has been possible to record the rapid increase in the number of new infections in a strip-by-strip plantation of *C. macrocarpa* and *C. lusitanica* at South Kinangop (Fig. 4A, B). Between March 1950 and March 1952 the total number of infections in *C. macrocarpa* rose from 28.7 to 97.5%, in *C. lusitanica* from 9.0 to 61.1%. In this plantation a very large amount of inoculum was being disseminated at an age when the trees were most susceptible, and the above figures can be contrasted with the 1945-6 planting of *C. macrocarpa* where the percentage of trees infected has only reached 78.6. In March 1950 counts were made in plantations of *C. macrocarpa* adjacent to the 1948 planting, the number of trees infected being 98.0% in 1946 planting, 46.4% in 1942 and 6.0% in 1933. 1949

planting showed 6.0% infected in March 1950, and subsequent counts have indicated that infections in this plantation are increasing at the same rate as the 1948 planting, with a year's difference in the total number infected.

Trees appear to enter the period of maximum susceptibility during the second year of growth in the plantation, and the susceptibility may fall again after the third year, although the climate and general growing conditions will also have an effect. Trees in the first year of growth are not particularly susceptible, and the same is true of very small branches on plantation trees and hedges. Wagener (1948) noted that hedges were comparatively free of infection by *Coryneum cardinale*, and



Text-fig. 4. The progress of canker disease in sample plots, 1948 planting.

suggested that this was due in part to the slower growth rate of the host. It seems likely either that infections dry out and thus do not progress in the thin bark tissues of young trees, small branches and hedges, or that the small concentrations of mobile food substances available to the fungus are not sufficient to allow it to become established except under the most favourable conditions of moisture.

The height of a tree has an effect on its chance of becoming infected since conidia fall on liberation. Older trees are less susceptible, since the chances of conidia being carried up into the higher branches, from infections at a lower level, are not great, whilst conidia from cankers actually in the crowns of tall trees would soon fall to a level where the cork cells of the bark prevented infection.

Recovery in *Cupressus macrocarpa* appeared to be more complete in the 1945-6 planting than in the 1948 planting, where the heavy rainfall of March, April and May 1951 resulted in renewed fungal activity in a large number of 'recovered cankers'. It was not possible to study recovery in the 1931 trees.

Recovery in *C. lusitanica* was normally complete, and renewed fungal activity was only recorded in a few of the infected trees kept under observation.

Effect of host species

The lower susceptibility and higher recovery rates of *C. lusitanica* as compared with *C. macrocarpa* have already been noted. Where the amount of inoculum is very great as in the 1948 strip-by-strip planting of the two species (Text-figs. 4A, B) infection in *C. lusitanica* may reach 61.1 %, but where most of the infected *C. macrocarpa* has been removed or is distant from the *C. lusitanica*, the percentage of trees infected is not higher than 22.0, and tree counts recently made on Mt Kenya, where all infected *C. macrocarpa* has now been removed, in 1948-planted *C. lusitanica* showed only 2.5 % infection.

Effect of climate

All the recorded plots have been within the area covered by two rainfalls annually and where ground mists are normal and frequent during July and August.

Map 2 shows that many of the early records of the disease were received in July and August—months when the disease is particularly active in new infections initiated from conidia formed a few weeks after the start of the rains. This is borne out by the sample plot recording, where the maximum number of new infections has been observed between June and September.

Although new infections are apparent within 5 weeks, renewed activity in old infections is often delayed for longer periods, and consequently a higher number of recovered trees was sometimes recorded in June than for the previous dry season recording. Otherwise, the number of recovered trees was always highest during the dry season but fell again after June and remained low until the end of the short rains, in January. This is in contrast with conditions west of the Rift Valley, where cankers are active and acervuli formed only during the rains.

The number of trees with two cankers or more sometimes showed a steeper rise to the September recording (Text-fig. 3B) than for first infections, and this might be due to the fact that although further infections may occur on the same tree the calm weather conditions of the mist season do not promote dissemination of conidia and infection of distant trees.

The percentage of trees with one canker fell, in comparison with the percentage with two cankers, by the time that a large proportion of the trees in a plot had been infected (Text-fig 3A, B and Text-fig. 4A). Similarly, the percentage of trees with both one canker and more than one might apparently fall as the number of trees killed in a plot began to rise steeply (Text-fig. 4A).

The largest number of trees killed was recorded during the dry season. At this time heavily diseased tissue tends to dry out and crack, and ring-barking by the canker thus becomes complete. Further, the increased water uptake during the dry season may be interfered with where the sapwood has become infected.

Effect of silviculture

Pruning. Although it is apparent that discernible wounds are not essential for infection, numbers of new infections are initiated through pruning scars or wounds, especially when pruning is undertaken at a season of the year when conidia are being produced. In Text-fig. 3 B the two plots were pruned in January 1950. In March 1950 there were 100 cankers and in August 1950, 276, of which eighteen had been started through pruning wounds. Seventeen new infections through pruning wounds were recorded for the plots shown in Text-fig. 3 A during the same period. New infections through pruning wounds in *C. lusitanica* have been recorded rarely.

In two plots of 1946-planted *C. lusitanica* left unpruned, three new cankers were recorded on the extremities of the lowest branches in March 1952. Presumably, the conditions of high humidity and reduced air movement resulted from delayed pruning, allowing infections to take place in the young growing parts of branches.

EXTENSION OF CANKERS IN TREES

Sample plots

The length and breadth of a number of cankers have been measured regularly in sample plots. Measurements could not be made with great accuracy, since it was usually difficult to determine the extent of a canker without disturbing the bark tissues.

There is considerable variation in the rate of extension of cankers at different seasons, in different species and even in two cankers on the same tree. The rates of extension of fourteen natural cankers are shown in Table 6.

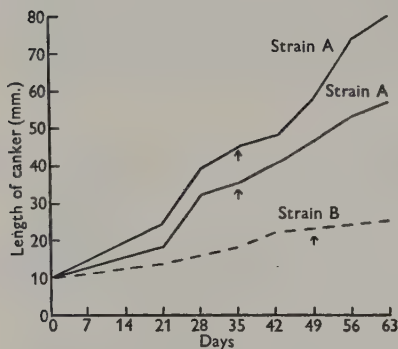
TABLE 6. *Extension of natural cankers in Cupressus macrocarpa and C. lusitanica*

Canker no.	Host species	Interval between measurements in weeks	Increment per week (cm.)	Interval between measurements in weeks	Increment per week (cm.)
Longitudinal:					
1	1945 <i>C. mac.</i>	9 (June–Aug.)	0·33	20 (Aug.–Dec.)	0·05
2	1945 <i>C. mac.</i>	9 (June–Aug.)	2·11	—	—
3	1946 <i>C. mac.</i>	9 (June–Aug.)	0·11	—	—
4	1946 <i>C. mac.</i>	9 (June–Aug.)	0·22	—	—
5	1946 <i>C. mac.</i>	9 (June–Aug.)	0·55	—	—
6	1946 <i>C. mac.</i>	9 (June–Aug.)	0·11	—	—
7	1948 <i>C. mac.</i>	20 (Jan.–May)	0·55	20 (Aug.–Dec.)	0·35
8	1948 <i>C. mac.</i>	20 (Jan.–May)	0·65	20 (Aug.–Dec.)	0·10
9	1945 <i>C. lus.</i>	15 (Oct.–June)	0·33	—	—
10	1945 <i>C. lus.</i>	15 (Oct.–June)	0·47	—	—
11	1945 <i>C. lus.</i>	24 (Jan.–June)	0·21	—	—
12	1946 <i>C. lus.</i>	24 (Mar.–Sept.)	0·17	—	—
13	1947 <i>C. lus.</i>	14 (June–Sept.)	0·32	—	—
14	1947 <i>C. lus.</i>	14 (June–Sept.)	0·29	20 (Sept.–Jan.)	0·14
Lateral:					
1–8	<i>C. mac.</i>	—	Range 0–0·44		
9–14	<i>C. lus.</i>	—	Range 0–0·13		

Inoculated trees

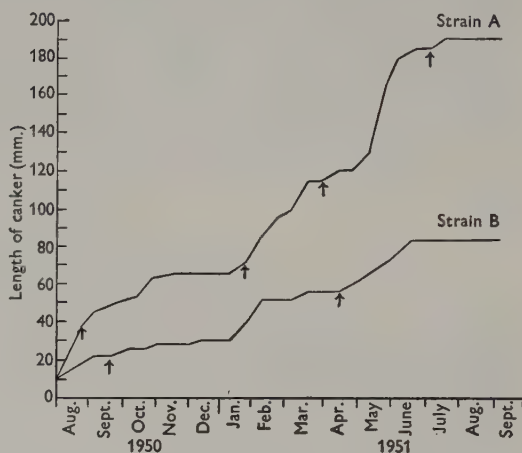
Trees were inoculated by placing standard disks of mycelium on agar into a vertical slit 1 cm. long penetrating to the wood. The slits were then sealed for 3 weeks with 1 in. Sellotape.

Text-fig. 5 shows the rate of extension in a typical strain B and two typical strain A cankers on 1-year-old *C. macrocarpa* inoculated in April shortly after the



Text-fig. 5.

Text-fig. 5. The longitudinal extension of inoculated cankers in 1-year-old *C. macrocarpa*. Arrows indicate when ripe acervuli were present.



Text-fig. 6

Text-fig. 6. The longitudinal extension of inoculated cankers in 2-year-old *C. lusitanica* throughout one year. Arrows indicate when ripe acervuli were present.

beginning of the rains. The rate of extension with a strain A canker builds up for 4 weeks, after which there is a falling off associated with the formation of acervuli. By the sixth, seventh or eighth week after inoculation the canker may start to elongate again if conditions are suitable. With strain B the progress is much more slow and ripe acervuli may not be present until the eighth week.

Text-fig. 6 shows the extension of two cankers on 2-year-old *C. lusitanica*, one strain A and one strain B, throughout one year. During the months of September–November extension is slow, but following the short rains, with a lag phase of up to 1 month, there is more rapid extension. There may or may not be a falling off in the rate just prior to the long rains, but rapid extension starts again during the rains with the usual lag phase. During July, August and September extension may cease altogether, as happened in 1951 with the two cankers of Text-fig. 6 although there were heavy ground mists.

Table 7 shows the detailed results of tree inoculations carried out at different times of the year on *C. macrocarpa* and *C. lusitanica* of various ages at Hombe.

The rate of extension with trees inoculated in January was slower than those inoculated in November or December during the rains. Differences between age classes were not significant. The differences between species will be discussed in another paper.

Where further infections occur on a tree, the initial extension of the second and subsequent cankers is always more rapid than the extension of the original infection and there is no pronounced lag phase.

TABLE 7. *Effect of time of inoculation and age of tree on extension of inoculated cankers at Hombe*

Host	Age of host in years	Date of inoculation	Mean length of canker after 35 days in mm. with standard error
<i>C. macrocarpa</i>	2	1 Nov. 1951	37.45 \pm 2.613 (20)
	2	7 Jan. 1952	17.75 \pm 1.297 (12)
<i>C. lusitanica</i>	3	29 Dec. 1950	24.14 \pm 1.048 (50)
	5	29 Dec. 1950	23.66 \pm 2.422 (27)
	2	1 Nov. 1951	25.33 \pm 2.090 (15)
	2	7 Jan. 1952	21.38 \pm 2.017 (13)
	6	7 Jan. 1952	21.00 \pm 2.597 (13)

Figures in brackets are numbers of cankers measured.

THE ORIGIN OF THE DISEASE

The problem presented by the *Monochaetia* canker disease of cypresses in East Africa is similar to that of the *Coryneum* canker of cypresses in California first reported by Wagener in 1928. The almost identical pathology of the two diseases has been commented on by Wagener (1948). Wagener, however, was concerned with the appearance of a serious disease on an indigenous species in plantations caused by a previously undescribed fungus, whereas in East Africa one is concerned with a disease on an exotic host species caused by a fungus not previously recorded in this country—indeed, it is probable that no species of *Monochaetia* has previously been recorded in Africa (Natrass & Ciccarone, 1947). Wagener (1948) came to the conclusion that the fungus had probably been imported from abroad, though there remained the possibility of the mutation of a pathogenic species of *Coryneum* from a saprophytic one, or that *C. cardinale*, the pathogen, had in fact been present as an unrecorded indigenous species and had become serious as a parasite with the extensive planting of cypresses.

The situation in East Africa is rather more complex. The perfect stage of *Monochaetia unicornis* has been found and the imperfect stage has also been found on an indigenous host (*Juniperus procera*) in a 'strain' pathogenic on cypresses and in another 'strain' not pathogenic on cypresses. The exact status of the latter 'strain' has yet to be determined, as has also the weakly pathogenic 'strain B', the distribution of which appears to be limited by ecological conditions.

Monochaetia unicornis is also present, though not as a serious parasite, in South

Africa, and it has been found causing cankers in Southern Rhodesia. A species of *Coryneum*, very probably *C. cardinale* Wagener, was received by the writer from Dr Talbot in Pretoria. This fungus produced a canker under laboratory conditions, but there have been no records of serious outbreaks of the disease from South Africa. Similarly, *Monochaetia unicornis* has been reported in America (Natrass & Ciccarone, 1947) but does not cause a serious disease there.

The earliest date when the *Monochaetia* canker first caused infections was 30 years after the first planting of cypresses in East Africa and 10 years after the first large establishment of plantations. Strain B might well have been active but not observed before this time.

The records of the disease would suggest very strongly that it started from a single focus, probably Shume Magamba in Tanganyika. There are stands of indigenous *Juniperus procera* in this area. The presence of collections of *Monochaetia* on *Juniperus* now may represent a migration from infected cypresses, although this seems unlikely in the case of the strain non-pathogenic to cypresses. The alternative initial focus for the disease is Machakos, but there is no known record of *Juniperus* in this area, although the other East African coniferous genus *Podocarpus* is present (*P. gracilior*).

Assuming the spread of the disease from a single focus one might well postulate the evolution of a parasitic strain of *Monochaetia unicornis* from a strain only mildly pathogenic on *Juniperus*, especially when it is considered that an increased growth rate is apparently the only factor required to produce active parasitism. Nevertheless, the possibility of the introduction of a pathogen from abroad remains.

I should like to acknowledge the assistance which I have received in this investigation from Dr Natrass and from the Conservators and staffs of the Forest Departments of Kenya, Tanganyika and Uganda. Particularly, I am indebted to the Divisional Forest Officer and Foresters of the Nyeri Forest Division in Kenya where my field laboratory was established.

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EXPLANATION OF PLATE 3

- Fig. 1. Resin oozing from a new canker infection at a branch crotch in 5-year-old *C. lusitanica*.
Fig. 2. A 3-year-old *C. macrocarpa* tree in which the leader has broken off as a result of girdling by a canker.
Figs. 3, 4. Wing-like growths associated with stem cankers. In fig. 4 the canker has been cross-cut at the branch crotch where infection originated and extensive growth rings can be seen on the unaffected side of the stem.
Fig. 5. A twisting effect produced by two stem cankers on 5-year-old *C. lusitanica*.

(Received 30 August 1952)

VERTICILLIUM WILT OF THE HOP

VI. THE RELATIVE ROLES OF ROOT AND STEM IN THE DETERMINATION OF WILT SEVERITY

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(With Plate 4)

The two hop varieties Fuggle and OR 55 have respectively low and high resistance to wilt caused by virulent strains of *Verticillium albo-atrum*. When the pathogen enters through the roots it grows vigorously in the stems of Fuggle plants and slightly in the stems of OR 55 plants. Leaf symptoms are correspondingly severe and mild.

If Fuggle stems are grafted on to OR 55 roots and *vice versa*, and the fungus thus invades the stems from roots of the opposite resistance type, it now grows slightly in the Fuggle stems, and vigorously in the OR 55 stems. Leaf symptoms are correspondingly mild and severe, and thus wilt intensity is related to the variety of root and not to the variety of stem. Evidence is available which shows that this is not due to the transfer of a 'resistance factor' generated in the resistant roots.

The variety Fuggle has low resistance to virulent strains of *V. albo-atrum* but high resistance to mild strains. As in the case of varietal type this resistance is expressed by vigorous or slight growth of the fungus in the stems (and corresponding leaf symptoms) when the fungi are introduced through Fuggle roots. When they are directly inoculated into the stems there is no differentiation of the strains. If a small inoculum is used both strains induce mild wilt. If a large inoculum is inserted into a pith cavity both strains induce severe wilt. The reaction of the Fuggle stem is thus governed by conditions relating to the type of invasion and not to the strain of pathogen.

It is suggested that this also applies with root inoculations. Normal stems of both Fuggle and OR 55 varieties have a high resistance to invasion from a weak source of the pathogen (such as exists in a highly resistant root), and the fungus can invade the stems heavily only from a suitable inoculum source (i.e. a root of low resistance).

Heavy invasion of the stem from a root may be due to

- (1) A lowering of stem resistance by:
 - (a) toxins diffused from the invaded root,
 - (b) an impairment of normal root function.
- (2) An increase in invasive power of the pathogen by:
 - (a) the establishment of a 'food base' in the root,
 - (b) the diffusion of a 'fungal accelerator'.

The main conclusions that the primary focus of the disease is in the root and that the normal stems of all varieties possess a similar (and possibly high) resistance is supported by other researches on *Fusarium* wilt of tomatoes and other wilt diseases and may be of general application.

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INTRODUCTION

The studies to be described herein provide some evidence on the mode of invasion of the hop plant by the vascular pathogen *Verticillium albo-atrum*. Researches by other workers on *Fusarium* wilt of tomato will also be considered, since they give similar evidence and suggest that the broad conclusions which can be drawn may be of application to other vascular wilt diseases.

The experiments on hop wilt were made separately at various times since 1944. The correlation between the various results was unsuspected until recently, and thus many experiments necessary for the full development of the work have not been made. Even so it is thought advisable that the available evidence should be published now, so that the conclusions based upon it can be tested by other workers. The evidence from previous work must first be considered.

(a) *Correlation between stem invasion and leaf symptom intensity*

Verticillium albo-atrum normally invades the hop plant in a similar fashion to most other wilt pathogens, viz. through the roots. Thence it grows through the xylem of root and stem and causes leaf symptoms. It has been suggested by Gäumann (1950) that such symptoms (of many wilt diseases) are caused by toxins which are carried 'far ahead' of the advancing mycelium in the sap stream. This suggestion may imply that such toxins can be formed in the roots and affect the leaves without stem invasion occurring. Although the leaf symptoms of hop wilt (yellowing and necrosis without loss of turgor) suggest toxic action rather than plugging of the vessels, the evidence from plant invasion studies indicates that the toxins are probably *not* produced solely in the roots. This also applies to tomato *Fusarium* wilt (Dimond, Davis, Chapman & Stoddard, 1952; Keyworth & Dimond, 1952).

Leaf symptoms of hop wilt and tomato wilt appear only after the stem has become invaded and usually only when the fungus has grown almost or quite to the level of the leaf in question. Of perhaps even greater significance is the fact that the extent of lateral growth of *Verticillium* in the hop-stem xylem (i.e. whether it is confined to sectors or invades the whole xylem tissue) also affects symptom intensity.

Although it may be argued that stem invasion is purely secondary, it is considered by the writer that this is not so and that active stem invasion is a necessary prerequisite for the full expression of leaf symptoms. Although toxins may cause these symptoms they are probably formed in the stem as well as in the roots and may diffuse in wilting concentrations for only short distances ahead of the advancing fungus. The stem cannot therefore be regarded simply as an inert channel for toxin diffusion.

(b) *Correlation between resistance of the hop plant and stem invasion*

The experiments and conclusions to be reported deal largely with the effects of host resistance on stem invasion. This study is complete in itself, and the evidence

can be interpreted strictly in this sense. For the reasons already given, however, leaf-symptom intensity is thought to be directly related to the extent of stem invasion. Evidence of such symptoms is therefore given as supporting that on stem invasion and also because these symptoms constitute the end-point of the wilt process, in which stem invasion is an essential stage.

Two, probably related, forms of host resistance are considered herein, viz. (a) the resistance of one hop variety to two races of *V. albo-atrum* of differing pathogenicity and (b) the resistance of two or more varieties to one race of *V. albo-atrum*.

Isaac & Keyworth (1948) showed that there were two races of *V. albo-atrum*. One (virulent) caused the Progressive form of hop wilt and the other (mild) caused the Fluctuating form. They were distinguished by growing plants of the Fuggle variety in soil inoculated with either race of fungus. The plants showed severe symptoms when thus root-inoculated with the virulent race but only mild symptoms when root-inoculated with the mild race.

Keyworth (1947) reported that hop varieties differed in their reaction to the virulent race of *V. albo-atrum* when inoculated through their roots. Whereas the Fuggle variety showed severe symptoms and had low resistance, other varieties (e.g. OR 55) showed mild symptoms and had high resistance.* The symptoms caused by the virulent race in OR 55 plants were very similar to those caused by the mild race in Fuggle plants. The resistance of OR 55 to the virulent race was thus of the same order as that of Fuggle to the mild race.

The expression of high or low resistance was as follows:

If resistance was low (Fuggle infected through its roots with a virulent strain) the fungus grew vigorously in the stem xylem, most of the vessels becoming invaded and/or discoloured. The leaves in succession from the base of stem showed severe symptoms as the fungus grew up the xylem, and eventually the stem was killed. The fungus meanwhile grew out of the xylem into the moribund stem and leaf tissue.

If resistance was high (Fuggle, root-infected with a mild strain, or OR 55, root-infected with a virulent strain) the effect was strikingly different. The fungus grew fairly extensively up the stem but its lateral growth in the xylem was restricted, and it was confined to sectors or to the centre of the wood cylinder. New uninvaded xylem tissue was often formed. Leaf symptoms were mild and usually consisted of sectorial yellowing and necrosis on a few leaves.

Few detailed observations were made on the roots of these differently invaded plants. It was found, however, that if the plant was showing low resistance its roots became moribund and died, usually with extensive fungal invasion. Conversely, if the plant was showing high resistance its roots remained apparently almost normal

* The varieties were described as susceptible and resistant. For reasons given later (see 'Researches on other wilt diseases') this description is not in accordance with later definitions now accepted by the writer. These terms are replaced herein by 'low resistance' and 'high resistance'.

and grew. They were not so extensively invaded by the pathogen (see also 'Grafting experiment').*

The experiments described above probably involved multiple infection of the roots. In one other experiment on the effect of the two races on the Fuggle variety single root inoculations were made by the insertion of a drop of spore suspension, or agar bearing the fungus, into a wound. The effects of these small-sized single inocula were identical with those of the multiple inoculations. This suggests that the size of the inoculum did not affect the result of root inoculation. This finding should be compared with those for stem inoculation (see below).

(c) *Former conclusions on the distribution of resistance in the plant*

If, after root inoculation, plants of one variety showed high resistance and those of another variety showed low resistance, this differential resistance was evident in the differential invasion of the xylem tissue *throughout the whole of each plant*. In particular, it was apparent that the stems were invaded to different extents.

It was thus concluded by the writer that in the xylem of a plant of low resistance the fungus encountered a medium of uniformly low resistance, extending throughout root and stem, through which it grew uniformly and actively. Conversely in a plant of high resistance the xylem tissues of root and stem presented a medium of uniformly high resistance in which the fungus made only slight growth. If the part played by the stems in this supposed effect is considered it is apparent that a necessary corollary to the conclusion is that the stems differ *intrinsically* in their resistance to invasion. Thus it should be possible to detect these differences in normal healthy stems.

Such differences have, in fact, been sought by workers on other wilt diseases. This is regarded by the writer as evidence that the conclusions may have been widely accepted (although apparently not stated).

Thus many workers have assessed the reaction of the normal healthy stems of varieties of high and low resistance, to toxins formed by wilt organisms. Most of these studies have shown, however, that such stems possess equal sensitivity to these toxins (e.g. Dowson, 1922; Gäumann, St Naef-Roth & Miescher, 1950). Other workers have attempted to correlate plant resistance with chemical constitution. Irving, Fontaine & Doolittle (1945) report the presence in tomatoes of a compound, Lycopersicin (now called Tomatin), which suppresses the growth of *Fusarium oxysporum lycopersici*. They used normal complete plants of varieties of high and low resistance and found almost equal amounts of the compound in both types. The bulk of each plant was presumably composed of stem and leaf.

These chemical studies, which attempt to assess stem resistance without growth of the pathogen, suggest that perhaps the conclusions formerly reached by the writer are incorrect, i.e. stems of varieties of differing resistance do *not* differ

* How much of these effects was due to direct fungal growth in the roots and how much to the health of the stems was not determined.

intrinsically in their reaction to invasion. Chemical investigations are open to the criticism, however, that the compounds studied may not be responsible for the effects observed after natural infection. More conclusive evidence can be obtained by studying the effects of fungal invasion of different stems under uniform conditions.

If the requirements of such tests are considered it becomes apparent that when varieties of differing resistance are infected through their own roots their stems are not invaded under uniform conditions. This is because (*a*) they are growing on roots of different varieties and (*b*) these roots are differently invaded. Uniform stem invasion conditions can be obtained, however, by (*a*) testing the reaction of different stems invaded through roots of one variety, and (*b*) inoculating the stems of one variety directly with the two races of *V. albo-atrum* to which plants of this variety show differential resistance when root-inoculated.

These tests have been made and are described below. An experiment similar to the grafting experiment to be described was reported by Heinze & Andrus (1945). Their results, which have a considerable bearing on the present study, are considered later.

GRAFTING EXPERIMENT

The object of this experiment, made in 1950, was to test the reaction of stems of varieties of high and low resistance, when invaded through roots from varieties of (*a*) low resistance and (*b*) high resistance. Stems and rootstocks from plants showing high (varieties OR55 and OM26) and low (varieties Fuggle and R2/25*a*) resistance to wilt following root inoculation with virulent strains of *V. albo-atrum* were grafted to give the four possible combinations of high and low resistance in the composite plants. The numbers of plants available were as follows:

Resistance category of scion variety	Resistance category of rootstock variety	No. of com- posite plants
High	Low	30
Low	Low	23
High	High	32
Low	High	27

These plants were set out in the field in non-randomized plots at the end of April. Infection was from 20 g. of chopped hop stem, naturally infected with a virulent strain of *V. albo-atrum* which was put into each planting hole.

Each plant was restricted as far as possible to one stem, but laterals from this stem were allowed to grow. Wilt symptoms were first seen on a few plants on 27 June, but extensive wilting did not occur until mid-July. Records were made on 28 July, 22 August, 7 and 27 September. The varieties Fuggle and R2/25*a* reacted similarly to infection and also the varieties OR55 and OM26. Varietal distinctions within each resistance category are thus ignored and the varieties described as either Fuggle (typical low-resistance) or OR55 (typical high resistance). The first and last records are given in Table 1.

TABLE 1. *Wilt symptoms on grafted plants*

Scion variety	Root-stock variety	No. of plants	28 July Nos. in each symptom category*					27 September Nos. in each symptom category*				
			D	W+	W	MW	H	D	W+	W	MW	H
OR55	Fuggle	30	2	14	6	4	4	30	—	—	—	—
Fuggle	Fuggle	23	1	11	3	5	3	23	—	—	—	—
Fuggle	OR55	32	—	—	4	5	23	3	1	3	10	15
OR55	OR55	27	—	—	2	2	23	1	—	1	3	22

* Symptom categories:

D = all leaves dead.

W+ = plants nearly dead but with a few normal leaves at the tips of the main stems or on laterals.

W = many leaves showing severe symptoms.

MW = a variable number of leaves showing mild symptoms (or a few showing severe symptoms).

H = leaves normal in appearance.

From these results it is apparent that all the scions (i.e. both OR55 and Fuggle) on Fuggle rootstocks showed severe wilt and were dead by 27 September. On the other hand, most of the scions (OR55 and Fuggle) on OR55 rootstocks showed mild symptoms throughout the season. The OR55 scions were somewhat less affected than the Fuggle scions however.

The intermediate symptom records showed that many of the scions on OR55 rootstocks became invaded by the fungus at an early date. The milder symptoms apparent at the end of the season were thus not due to late invasion of the scions because of slower growth of the fungus through the OR55 rootstocks but to the reduced activity of the fungus in the scions.

The differences between the group of plants, both in respect of wilt incidence and of stunting caused by the disease, were very apparent on the plot. The OR55/OR55 group were very vigorous with stems of an average height of over 11 ft. on 22 August. The Fuggle/OR55 group were somewhat less vigorous but had an average height of 10 ft. The OR55/Fuggle and Fuggle/Fuggle groups were both stunted to about the same extent and were of average height 7 ft. These differences are illustrated in Pl. 4, fig. 1.

The extent of invasion of representative scions in each group was determined at the end of the experiment by isolation tests. The main stems of the selected plants were cut 3 in. above ground level and portions taken at 6 in. intervals above this were incubated in moist chambers (Keyworth, 1951). The extent of invasion of the rootstocks of the five Fuggle/OR55 and the four OR55/Fuggle plants used in the stem tests was also studied. The OR55 and Fuggle rootstocks were markedly different in size (Pl. 4, fig. 2). The OR55 rootstocks were quite normal in external appearance, but the Fuggle rootstocks were discoloured and bore many completely dead roots. Because of the difficulty of testing the whole of the OR55 root systems for the presence of the fungus four or five typical roots were selected for test

from each plant. Owing to their smaller size it was usually possible to test the whole of each Fuggle root system. The actual lengths of root tested from each type of plant were thus similar. The roots were washed and surface-sterilized with mercuric chloride. Portions were then taken at 2 in. intervals and incubated in moist chambers, later being observed for growth of the fungus from the xylem tissue. The results of the stem and rootstock tests are summarized in Table 2.

TABLE 2. *Presence of Verticillium albo-atrum in roots and stems of grafted plants*

Plant no.	Scion	Root-stock	Symptom category	Roots			Stem		Notes on invasion of stem xylem
				Length examined (in.)	% Length invaded	% Length dead	Length	% Length invaded	
1	OR 55	Fuggle	D	52	77	11	91	100	Heavy and complete
2	OR 55	Fuggle	D	163	68	8	163	100	Heavy and complete
3	OR 55	Fuggle	D	103	100	22	103	100	Heavy and complete
4	OR 55	Fuggle	D	90	92	36	187	100	Heavy and complete
5	Fuggle	Fuggle	D	—	—	—	67	100	Heavy and complete
6	Fuggle	Fuggle	D	—	—	—	139	100	Heavy and complete
7	Fuggle	OR 55	W	54	15	0	115	100	Heavy and complete
8	Fuggle	OR 55	MW	90	67	0	121	100	Slight and sectorial
9	Fuggle	OR 55	MW	109	55	0	139	78	Slight and sectorial
10	Fuggle	OR 55	H	117	0*	0	121	69*	Slight and sectorial
11	Fuggle	OR 55	H	146	2	0	157	0	—
12	OR 55	OR 55	MW	—	—	—	127	100	Slight and sectorial
13	OR 55	OR 55	MW	—	—	—	157	73	Slight and sectorial
14	OR 55	OR 55	H	—	—	—	181	57	Slight and sectorial
15	OR 55	OR 55	H	—	—	—	163	0	Slight and sectorial

* The apparently anomalous result may be due to the fact that the whole of the root system was not examined.

These results indicate that in most of the Fuggle scions growing on OR 55 rootstocks the fungus was confined to sectors of the xylem. It has already been seen that the symptoms were slight, and it is thus apparent that under these conditions the Fuggle scions were showing mild wilt and could tolerate the presence of the pathogen even though it was a virulent strain. They were thus reacting in the same way as OR 55 scions on OR 55 roots. Conversely the OR 55 scions on Fuggle roots could not tolerate the presence of the pathogen and reacted similarly to Fuggle scions.

It will be seen that the composite plants showed the same homogeneity of reaction to the fungus (i.e. root and stem both lightly or heavily invaded) as did plants growing on their own roots. The stems were therefore not reacting according to their varietal type but were merely reacting in the same fashion as the roots. On the other hand, the roots were reacting in accordance with their varietal type irrespective of the variety of scion they bore.

STEM INOCULATIONS

Few tests have been made of direct stem inoculation of varieties of differing resistance, but several experiments have been carried out in which the stems of one variety (Fuggle) were directly inoculated with virulent and mild strains of the pathogen. Since the Fuggle variety is highly resistant to one strain and has only low resistance to the other, these tests provide information of a similar type to that which would be obtained by inoculating two varieties with one strain. Two methods of inoculation were used.

(a) Method no. 1

These tests were made during the studies on fungal virulence (Isaac & Keyworth, 1948). A drop of spore suspension was injected with a hypodermic syringe directly into the xylem tissues of the stems. In most cases the amount that could be injected was very small owing to the solidity of the woody tissue. The inoculations were made in early June on Fuggle plants of two ages (1 and 4 years) and at heights of 9 in. and 3 ft. from ground-level respectively.* Similar results were obtained with the plants of both ages and in experiments done in two successive years.

Two virulent and two mild strains of pathogen were each inoculated into some 30-40 plants, and observations made on the extent of discoloration of the xylem and on the leaf symptoms produced. All the fungi grew upward in the xylem and discoloured it, often eventually to a height of several feet. Very little downward growth occurred from the point of inoculation, and the roots remained apparently healthy. The symptoms produced were in striking contrast to those produced by root inoculation with the same fungi. The virulent and mild strains all caused predominantly mild symptoms. Only a few leaves on each plant became yellow and sectorially necrotic, and the fungus grew sectorially in the xylem. A very few bines showed severe wilt, but the treatments did not differ markedly in this respect. The symptoms and progress of the fungi above the point of inoculation were similar to those following root inoculation with a mild strain.

(b) Method no. 2

Direct stem inoculations were made later by the writer (Keyworth, 1950) using a different method from that employed by Isaac & Keyworth (1948). One or more internodal pith-cavities were filled with a spore suspension of the fungus using a hypodermic syringe. The spore suspension was made up in a 0.5% agar solution to prevent it running out of the internodal cavity, and a hole was bored into the top of the cavity to allow the displaced air to escape. From 100,000 to 200,000 spores of either strain of the fungus were introduced into each internode.

* Other 'stem' inoculations referred to by Isaac & Keyworth were made into the previous year's stem bases on the 'sets' before planting. The true stems arose from buds on these stem bases and were thus not directly inoculated. In addition, the inoculum was placed close to both the old and new roots.

The method differed from that used by Isaac & Keyworth in several ways:

- (1) The number of spores was greater.
- (2) The inoculum was put into the pith-cavity instead of directly into the xylem, and the fungus presumably grew in this cavity before entering the xylem.
- (3) A considerable length of xylem tissue was uniformly exposed to subsequent invasion by the growing fungus (instead of the small unilaterally disposed area in the other method).
- (4) The inoculum was contained in agar. A few tests in which the spore suspension was made up in water and prevented from running out by rubber tape gave similar results to those using agar. This suggests that the presence of the agar, although convenient, was not obligatory.

In an experiment made in 1948, twelve Fuggle stems were inoculated (one internode) with a standardized spore suspension of a mild strain of the fungus and eleven stems with a similar spore suspension of a virulent strain. Records taken 7 weeks later showed that all the stems inoculated with either strain of the fungus were severely wilted and dead above the point of inoculation but were apparently healthy below this point. The symptoms and progress of the fungus above the point of inoculation were identical with those following root infection with a virulent strain. A further experiment made in 1949 confirmed this result. It also showed that the rate of wilting as measured by the number of affected leaves on four successive record dates at 4-day intervals was the same for both strains.

As with the other method there was again no difference between the effects of the mild and virulent strains. There was, however, a great difference between the effects of the two methods of inoculation. Method no. 1 gave typical mild symptoms and method no. 2 gave typical severe symptoms. The same differentiation as was obtained in stem symptoms by root inoculation with the mild and virulent strains was thus here obtained by the two inoculation methods irrespective of the strains used.

One test was made of the second method of inoculation on the stems of a highly resistant variety (no. 219). A virulent strain only was used and seven stems were tested. Of these four showed severe wilt and 3 showed mild wilt. The result was thus not conclusive but did demonstrate that stems of this variety could show severe symptoms when inoculated in this way.

DISCUSSION

The main results of the experiments can be summarized as follows:

- (1) The pathogen grows vigorously in the stems and causes severe wilt if:
 - (a) it invades the stem from the root of a variety of low resistance, irrespective of the stem variety;
 - (b) it invades the stem from a large inoculum source (in the pith cavity) irrespective of the strain of pathogen. This applies to Fuggle stems and may apply to other stems also.

(2) The pathogen grows slightly in the stem and causes mild wilt if:

(a) it invades the stems from the root of a variety of high resistance, irrespective of the stem variety;

(b) it invades the (Fuggle) stem from a small inoculum source (in the xylem) irrespective of the strain of pathogen.

(3) Stem reaction does not depend upon stem variety or the strain of pathogen (within the limits of the experiments) but only on the invasion source (i.e. the type of root or method of direct inoculation).

(4) Root reaction does depend both on root variety and on the strain of pathogen.

From (3) and (4) it follows that the *root* is the primary focus of the disease following natural infection, and that stem reaction is conditioned by factors operative in the roots.

This conclusion is borne out if the results are considered in terms of stem resistance (i.e. disease reaction). In the grafting experiment OR55 and Fuggle stems were grown under similar root-infection conditions (i.e. on infected OR55 or Fuggle roots). When under the same conditions their reaction was the same.* They thus showed equal resistance.

If these results are considered further two queries present themselves:

(1) *Was there any transfer of a 'resistance factor' from the OR55 roots to the Fuggle stems?*

This possibility has been considered in grafting experiments with other diseases, but no clear evidence has yet been obtained for the existence of such translocatable resistance factors. It is considered that none occurred in the present instance for the following reason. If a resistance factor was translocated from OR55 roots to Fuggle stems it must follow that no such factor was effective when the stems were grown on Fuggle roots. In the stem inoculation experiment, however, all the plants were grown on Fuggle roots. In spite of this the progress of the disease could be varied by the inoculation method, in one case the stems being invaded in a similar fashion to infection through OR55 roots. This, and other evidence from tomato-wilt studies (see below), suggests the absence of a resistance factor specific to OR55 roots. If this is so it must be presumed that the differences in stem reaction obtained on the different roots were related to the differential invasion of the roots.

* It should be noted that infection conditions on OR55 and Fuggle roots may be too dissimilar for slight differences in stem reaction to be detected by the recording methods available. There was an indication that when grown on infected OR55 roots the Fuggle and OR55 stems showed a slightly different reaction which an intermediate grade of infection might have revealed more precisely. Since the major differences in reaction which were obtained approximated to those prevailing under normal infection conditions, however, any major differences between the stems may be unimportant. The same arguments apply to the stem inoculations where the two methods of inoculation differed considerably.

(2) *If the stems of the OR55 and Fuggle varieties showed equal resistance was this resistance high or low?*

It might be considered that the OR55 and Fuggle stems were merely passive indicators of an action taking place solely in the roots—such as a diffusion of toxin (i.e. their resistance was equal because it was nil). The experimental evidence shows that this is not so.

For the reasons already stated (see Introduction) it is believed that the stems are not passive conductors of toxin but take an active part in the progress of the disease. If their invasion, either from roots or from directly inserted inoculum, is considered, it will be seen also that they cannot be regarded merely as inert media for the growth of the fungus. If this were so the pathogen would grow readily in them no matter how it was introduced. In the stem inoculations the fungi of either race were unable to make vigorous growth from the small inoculum. Before the fungus entered the xylem the stems were normal (i.e. growing on healthy roots). As the fungi grew they were apparently unable to establish conditions for vigorous growth and the stem remained near-normal. It may thus be concluded that the *normal* Fuggle stem (and presumably the OR55 stem also) possesses high resistance to this type of invasion by both races of the pathogen. When a large inoculum was put into a pith cavity, however, both races invaded the stems vigorously. This must have involved either some breakdown of stem resistance or an increase in the invasive power of the fungus.

By using the stem inoculation results to interpret the results of the root inoculations a tentative hypothesis on the mode of action of *V. albo-atrum* in the hop can now be formulated. In the small stem inoculations the virulent strain was unable to establish suitable invasion conditions and thus grew as slightly as the mild strain. In the large stem inoculations both strains were able to establish suitable invasion conditions and thus the mild strain grew as vigorously as the virulent strain. In root inoculations it may be postulated that the invasion conditions are determined in the roots.

When a hop root is invaded the interaction of the pathogen and root tissues is determined by the variety of the host and the pathogenicity of the race of fungus. Some root-fungus combinations (e.g. virulent strain in Fuggle root) will result in active root invasion. Others (e.g. virulent strain in OR55 root and mild strain in Fuggle root) will result in less active invasion. The stems are normally resistant to invasion, *whatever their variety*, and only where the root is first actively invaded is this resistance broken down (or the invasion power of the fungus increased) so that the stem also becomes actively invaded. In the other cases the fungus proceeds to a mild invasion of the stem which is able more effectively to exert its normal resistance.

The mechanism of this root effect is unknown. The following possible reasons for vigorous stem invasion would seem to warrant further study.

(a) *A lowering of stem resistance by the diffusion of toxin from the root.*

Gäumann (1950) states that toxic action precedes fungal invasion of the peripheral parts of the plant. He is referring to the wilt-producing toxins and does not give evidence in support of the statement. It is conceivable, however, that the toxins responsible for wilt symptoms (or some other precursory toxin) may, in concentrations too small to cause leaf symptoms, act upon the stem and lower its resistance. This would admit of the simple explanation that these toxins are not produced (or only formed in small amounts) by certain root-fungus combinations but are produced in quantity by others. Such an effect might depend of course merely on the extent of fungal growth in the roots. This explanation would also agree with the results from studies showing that the stems of varieties of differing resistance are equally influenced by equal amounts of toxin.

In this connexion the work of Irving (1947) on tomatin may also be significant. He reports that this compound, which may form the chemical basis of resistance to *Fusarium* wilt of tomatoes although found in varieties of both high and low resistance, is rapidly destroyed in the latter after fungal infection. This suggests the possibility that the diffused fungal toxins act directly on the compound or prevent its continued formation by the plant.

(b) *An increase in the invasive power of the fungus due to the establishment of a large inoculum*

As already noted, the greater fungal growth in the roots of low resistance may act merely through an increased toxin production. It is also possible, however, that there may be some 'food-base' effect such as has been noted with other fungi (e.g. *Fomes lignosus* attacking the roots of rubber trees (De Jong, 1933)). Such effects seem to act through a lowering of host resistance however.

Studies of the actual extent of fungal growth in the roots were made in the present study only in the grafting experiment. The observations of Snyder, Baker & Hansen (1946) on tomato *Fusarium* wilt indicate that in this disease the roots of low resistance contained more infected vessels than those of high resistance. The quantity of fungus *per vessel* was similar in both types of root suggesting that the difference between the roots lay in the ease with which the fungus could pass from one vessel to another. This possibility would seem particularly worth further study.

(c) *The diffusion of a fungal 'accelerator' from the invaded roots*

It is possible that the growth of the fungus in the root generates products which when diffused into the xylem elements of the stem, there encourage the growth of the fungus. Fontaine* reports that a compound has been isolated from tomato stems infected with *F. oxysporum lycopersici* which accelerates the growth of the fungus in culture.

* Private communication.

(d) *A lowering of stem resistance due to an impairment of normal root function*

The effect of fungal invasion of the roots on their normal functioning may play a part in the lowering of stem resistance. The observations of Harris (1936) that hop stems showed more severe symptoms when infected with a mild strain of *V. albo-atrum* if their roots were waterlogged support this suggestion. In the case of several wilt diseases (e.g. *Verticillium* wilt of the tomato) it has been shown that the production of fresh healthy roots may suppress wilt intensity. It is possible that this effect arises solely through an increase in water absorption. On the other hand, the stem inoculation experiments suggest that a stem growing on normal roots may tend to be more resistant than one on invaded roots. An increase in the healthy/diseased root balance may thus increase stem resistance.

RESEARCHES ON OTHER WILT DISEASES

The distribution, in the tomato plant, of resistance to *Fusarium* wilt has been studied by several workers. Some of the results obtained are very similar to those now reported for hops. The conclusions reached by the workers concerned differed, however, from those advanced by the present writer. The workers also differed from each other in their interpretation of the phenomena observed. It is now suggested that if the present interpretation of the hop studies is applied to the tomato work some of these differences can be resolved.

Before considering these studies, some explanation is necessary of a difference in terms used by the present writer and these earlier workers. The terms relating to plant resistance used herein follow the definitions suggested by the Plant Pathology Committee of the British Mycological Society (Anon. 1950). By these definitions a plant is *susceptible* if it can become infected. Since all the hop varieties studied by the author and all the tomato varieties studied in the other researches can be infected by their respective wilt pathogens, all are susceptible. They differ, however, in that after infection the pathogens exhibit differential growth and effects within them. According to definition, therefore, the varieties differ in resistance, some having high, and others low, resistance.

In the studies on tomato wilt, as in some other work on wilt diseases, e.g. Keyworth (1947), the term 'susceptible' has been applied only to varieties in which the pathogen grew actively and caused severe wilt under normal conditions. The term 'resistant' was used in an absolute sense to define a variety in which the pathogen grew less actively under the same conditions and caused only mild or no symptoms. For the reasons given, this usage is now considered by the writer to be incorrect. It is also thought likely that the description of a variety as 'susceptible' has given rise to the supposition that plants of such a variety (or any part of them) have no resistance whatsoever to the pathogen concerned. The influence of this supposition on the interpretation of resistance phenomena is apparent in the conclusions derived from the experiments described below.

Heinze & Andrus (1945) made stock-scion grafts of the tomato varieties Pan America (high-resistance) and Bonny Best (low-resistance), the composite plants then being root-inoculated with *Fusarium oxysporum lycopersici*. The results obtained were very similar to those now reported for hops. Both Pan America and Bonny Best stems became actively invaded and showed severe symptoms when inoculated through Bonny Best roots. When inoculated through Pan America roots the fungus made little or no growth in the stems of either variety and wilt symptoms were slight or absent. Since the Bonny Best stems were considered as normally 'susceptible' (i.e. as having low, or no resistance) a test was made to determine whether their freedom from disease when grown on Pan America roots was due to the transfer of a 'resistance factor' from these roots. Adventitious (Bonny Best) roots were induced on some such stems which were then inoculated through these roots. Although also supported on Pan America roots the stems became actively invaded from the adventitious roots and showed typical severe symptoms. It was thus concluded that they had acquired no resistance from the Pan America roots (i.e. that they retained their original supposed 'susceptibility'). Although not stated, it was presumably assumed that the other Bonny Best stems inoculated through Pan America roots were not actively invaded from them because of the confinement or slow growth of the pathogen in these roots.

The feature of the results which was regarded as the most outstanding was the so-called 'complete susceptibility' of the Pan America stems when inoculated through Bonny Best roots. Presumably on the grounds that no 'resistance-factor' was translocated from the Pan America roots this 'susceptibility' was regarded as intrinsic and unchangeable. Of the four plant parts involved (Pan America and Bonny Best stems and roots) three, viz. both the stems and the Bonny Best roots, were regarded as 'susceptible' and only one, viz. Pan America roots, as 'resistant'.

The conclusion was therefore derived that resistance was *confined* to the Pan America roots and was not transportable.

The interpretation of these results now suggested by the writer is as follows:

Pan America and Bonny Best stems have an equal and intrinsically *high* resistance. This resistance is lowered or overcome only when the pathogen invades the stems from Bonny Best roots (either basal or adventitious). When the stems are invaded from Pan America roots they retain their normal intrinsic resistance and fungal growth in them is slight. No transfer of a 'resistance factor' into either stem need therefore be postulated.

This interpretation supposes that a change can occur in the relative resistance of the stems—but in the opposite sense to that sought (but not found) by Heinze and Andrus. There is a *reduction* of resistance in stems inoculated through Bonny Best roots and not an increase of resistance in stems inoculated through Pan America roots. As noted above, it is thought likely that the present interpretation was rejected or not considered by Heinze and Andrus on *a priori* grounds if the

stems of the Bonny Best plants were thought to be unchangeably 'susceptible' (i.e. having no resistance whatsoever).

Snyder *et al.* (1946) disputed the conclusions advanced by Heinze and Andrus. They showed that the fungus made poor growth in Pan America stems growing on Pan America roots even when injected (as spores) into these stems through the cut end of the tap root. In Bonny Best stems on Bonny Best roots the fungus proceeded to active invasion. They thus showed that *under these conditions* the stems as well as the roots differed in their resistance. As noted elsewhere they also demonstrated a similar growth of fungus per vessel in both varieties, but a difference in the number of invaded vessels. The stem comparisons were, of course, made under different root invasion conditions, and, according to the present interpretation, the stems would be expected to react differently. There thus appear to be no irreconcilable differences between the two studies.

The latest study of this subject has been made by Scheffer & Walker (1952). Their conclusions are contrary to those both of Heinze and Andrus and the writer and they seek to show that major differences in resistance between tomato varieties occur in the stems as well as in the roots. Tomato cuttings were inoculated directly by means of spores sucked into their vessels (Keyworth, 1950). The cuttings were rooted in nutrient solution and potted into sand. 10–15 days after inoculation, wilt and vascular discoloration symptoms appeared in both high- and low-resistance varieties. At this time the fungus could not be isolated from the roots. The highly resistant varieties then recovered and grew normally but the low-resistance varieties showed progressively more severe symptoms. It was suggested that this difference between the varieties indicated a difference in resistance between their stems when grown under identical conditions. Scheffer & Walker give no evidence, however, on root invasion after the time when the stems showed similar symptoms. If the roots did become invaded the differential stem effect seen subsequently could have been related to the effects of the fungus in the roots. If this is not so some other explanation must be sought for the results of the grafting experiments. A further study of Scheffer & Walker's method would thus seem to be essential.

It should be noted here that the division of the experimental plants (tomato or hop) into stem and root may not be strictly accurate. The important distinction may be between aerial and subaerial parts of the plant. It is thus conceivable that any part of the plant below soil level, although anatomically a stem structure, may function as a root in respect of its reaction to the wilt pathogens concerned. This might provide a further explanation of Scheffer & Walker's results.

Another wilt disease to which the conclusions derived from the hop studies may be applicable is the Panama disease of the banana. Wardlaw (1935) reported that the pseudostem of plants of the susceptible Gros Michel variety could only be infected through roots.

Conclusion and suggestions for future research

The main conclusion, to be derived from the data presented, is that the stems of all the tested hop varieties have a similar and *positive* resistance to invasion by *Verticillium albo-atrum*. Whatever the variety, this resistance must be either reduced or overcome before active stem invasion can proceed. The roots of the different varieties differ in their resistance to fungal invasion after entry has occurred and the factors which determine varietal resistance are operative in the roots. The extent of invasion of the roots governs the extent of invasion of the stem. When active root invasion occurs (i.e. in a variety of low resistance), stem resistance is either reduced or overcome and active stem invasion can proceed. If less active root invasion occurs (i.e. in a variety of high resistance) stem resistance, relative to the invasion potential of the fungus in the roots, remains correspondingly high and less active stem invasion occurs.

The data on which these conclusions are based are incomplete and the conclusions are thus put forward solely as a basis for further study. It is considered of particular significance, however, that they can be applied to the results of other work on tomato wilt and probably other vascular diseases. They may therefore be of general application.

If correct, the conclusions may lead to a modification of previous views on the mechanism of action of wilt pathogens. In the present study the main emphasis has been laid on the extent of invasion of stems and not on the leaf symptoms produced. For the reasons already given it is thought, however, that the extent of stem invasion determines the severity of leaf symptoms. The progress of a wilt disease would thus appear to be in the nature of a 'chain reaction' in which the effects of root invasion govern the extent of stem invasion which, in turn, governs the severity of leaf symptoms. Although leaf symptoms may be caused by toxins diffused from the pathogen, stem invasion remains an essential link in the 'chain' and leaf symptoms cannot be caused solely by the action of the fungus in the roots.

It has been shown that attempts to differentiate between the normal stems of varieties of differing resistance, either in their reaction to toxins, or in chemical composition, have so far failed. If the intrinsic resistance of these stems is similar the reason for this failure becomes clear. The differences between the roots of different varieties would seem likely to repay intensive study, however, particularly to determine why some can become more actively invaded than others. The causes of the differential effects of this invasion on the stems should also be studied.

Several workers have shown that resistance to certain wilt pathogens can be increased by reducing the supply of nitrogen to the plant (Roberts, 1943; Keyworth & Hewitt, 1948). Keyworth & Dimond (1952) demonstrated that root injury increased the resistance of tomatoes to *Fusarium oxysporum lycopersici* and that this increased resistance was operative in the roots or stem base. This effect was considered to be nutritional in origin. The present study suggests that a nutritional

increase in plant resistance acts by affecting root invasion and not by modifying the stems. This possibility could be tested.

The chemotherapeutic approach to the control of wilt disease has probably been influenced by the supposition that a therapeutic compound should be introduced into the stem (as well as the roots) if it is to reduce the invasion of the stems.

If the present conclusions are correct, however, a reduction in the extent of root invasion alone will enable the stems to exert their normal resistance. The presence of the therapeutant in the stems may thus not be obligatory for wilt reduction.

Finally, it should again be noted that in the experiments described herein, and in the discussion, the division of the plant into stem and root has been adopted for convenience of description only, and may not be entirely accurate. The real distinction may lie between above- or below-ground parts of the plant, whether such parts are morphologically stem or root structure, or perhaps in some other unsuspected difference. The exact distinction between those parts of the plant which function in such different ways in relation to the pathogen remains a subject for further study.

The writer wishes to record his thanks to Mrs J. L. James (née Smith) and Miss P. M. Goode for their help in many of the experiments. Also to Dr R. V. Harris for his helpful criticisms of the work.

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Fig. 1

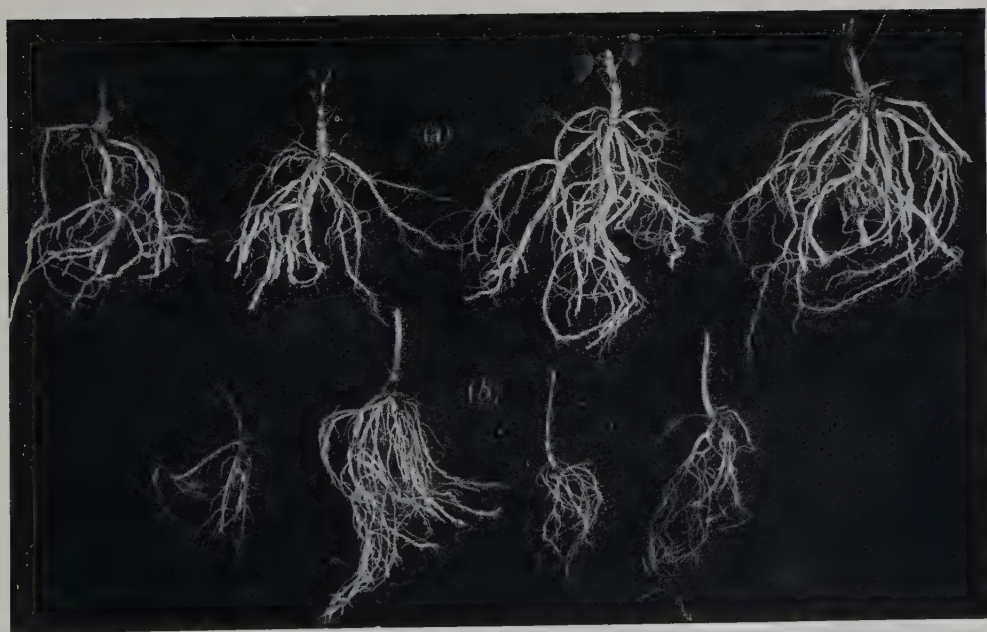


Fig. 2

KEYWORTH—*Verticillium wilt* of the hop

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EXPLANATION OF PLATE 4

- Fig. 1. Grafting experiment. Rows from left to right: 1. Fuggle/OR 55. 2. OR 55/Fuggle. 3. OR 55/OR 55. 4. Fuggle/Fuggle.
- Fig. 2. Roots of plants taken at random from grafting experiment. (a) var. OR 55. (b) var. Fuggle.

OBSERVATIONS ON FUNGICIDE CONTROL OF WITCHES' BROOM, BLACK-POD AND PINK DISEASE OF *THEOBROMA CACAO*

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Spraying experiments were started in Trinidad in 1940 and continued in Nigeria in 1950 to determine the control of certain cacao diseases obtainable by relatively frequent applications of copper fungicides, and also to test modified procedures designed to reduce spraying costs.

It was found that a spray applied only to the cropping regions of the trees reduced pod losses, which may include *Phytophthora* and *Diplodia* infections in addition to witches' broom disease (*Marasmius perniciosus*).

Experiments in Trinidad on witches' broom disease showed that monthly applications of 1% Bordeaux mixture reduced the incidence of both vegetative brooms and affected pods. The number of cushion brooms was not reduced; there was an apparent increase associated with spray applications to one particular cacao clone (ICS 4). There was no apparent advantage when the interval between spray applications was reduced to 1 or 2 weeks, because vegetative brooms occurred in spite of such frequent applications. However, if pod losses can be controlled directly, the occurrence of vegetative brooms would be unimportant.

Pink disease (*Corticium salmonicolor*) was completely controlled by applications of Bordeaux mixture. It appeared that there was at least a 12 months' residual effect from spraying.

Experiments were carried out in Nigeria on the control of black-pod disease (*Phytophthora palmivora*), using carbide Bordeaux, prepared by adding solid calcium carbide to 1% copper sulphate solution. Satisfactory disease control was obtained with applications at intervals of 1-4 weeks. Perenox (0.1%) was less effective than carbide Bordeaux. Knapsack spraying failed to protect all pods more than 7 ft. from the ground.

In Fernando Po, where Bordeaux mixture is used for the control of black-pod disease, two to four applications are made per season. Black-pod control is almost universal on plantations in Fernando Po, where there are consequently relatively few sources of infection.

WITCHES' BROOM DISEASE (*MARASMIUS PERNICIOSUS*)

INTRODUCTION

The control of witches' broom disease of cacao (*Marasmius perniciosus*) by the use of fungicides in combination with the removal of brooms was originally suggested by Stahel (1915) in Suriname, but was later (1932) found to be unsuccessful and was abandoned in favour of regular pruning and removal of brooms only. Experiments carried out by the Trinidad Department of Agriculture from 1932 to 1939,

with copper and sulphur fungicides*, applied both as sprays and as dusts, were not conclusive. Further experiments were therefore carried out on two estates in Trinidad in the period 1940-6.

SPRAYING PROCEDURES

There are various manifestations of witches' broom disease, which have been described and classified by Baker & Crowdy (1943). In the studies now to be described, it was convenient to record disease symptoms as either vegetative (fan and chupon) brooms, or cushion brooms, or affected pods. According to its age, a young tree which is not yet bearing any pods may produce only vegetative brooms, or both vegetative and cushion brooms. The three forms of the disease can occur simultaneously on a mature tree. These facts had to be taken into consideration in planning the experiments, because only knapsack-type pumps were available for the spraying work. This limitation made it impracticable to spray the tops of mature trees. Accordingly, young trees were sprayed completely for observations on vegetative and cushion brooms. In mature trees, fungicide was applied to the cropping regions only, for observations on the control of diseased pods and the results obtained are described separately.

RESULTS

Spray applications to entire trees

(i) *Monthly applications of 1% Bordeaux mixture versus no spray*

The trees at River Estate used for this experiment had been vegetatively propagated and comprised four Imperial College Selections, numbered ICS 4, 5, 20 and 97 respectively (Pound, 1934, 1935). The brooms were removed and counted at monthly intervals, vegetative and cushion brooms being recorded separately throughout the period of the experiment (May 1940 to February 1942). The results obtained are summarized in Table 1. In this experiment, the treatment effect is highly significant ($P < 0.01$) for vegetative brooms, but not significant ($P > 0.05$) for cushion brooms.

TABLE 1. *Effect of monthly applications of 1% Bordeaux mixture, expressed as mean number of brooms per tree*

	Type of broom	
	Vegetative	Cushion
Treatment: no spray	40.05	12.75
Bordeaux mixture	17.53	22.53
Difference	22.52*	9.78
Critical difference at 5 % point	10.89	—

* Difference significant at 5 % point.

The monthly applications of Bordeaux mixture were associated with a significant decrease in the number of vegetative brooms; the difference (22.52) exceeds the critical difference (10.89). There was an apparent but not significant increase in the numbers of cushion brooms (9.78) associated with spray applications. This anomaly is considered in detail below.

Some of the beneficial effect of Bordeaux mixture against vegetative brooms might be due to its shading effect. Murray (1940) has observed a midday drop in assimilation, with transpiration effects suggesting that shade favours the cacao leaf more than full sunlight. Accordingly, an additional spray treatment with an inert material was included in another experiment.

(ii) *Monthly applications of 1% Bordeaux mixture versus monthly applications of finely-ground limestone in water (12% by weight) with linseed oil (2% by volume) as an adhesive*

The trees at River Estate used for this experiment comprised the same four Imperial College Selections. The results obtained for the period May 1940 to February 1942 are given in Table 2. In this experiment, the treatment effect is highly significant ($P < 0.01$) for vegetative brooms, but not significant ($P > 0.05$) for cushion brooms.

TABLE 2. *Comparison of 1% Bordeaux mixture and of limestone applications for the control of vegetative and cushion brooms, expressed as mean number of brooms per tree*

	Type of broom	
	Vegetative	Cushion
Treatment: (a) No spray	35.60	9.63
(b) Limestone spray	27.35	8.85
(c) Bordeaux mixture	17.81	32.33
Difference: (a) v. (b)	8.25	0.78
(a) v. (c)	17.79*	22.70
(b) v. (c)	9.54*	23.48
Critical difference at 5 % point	8.42	—

* Difference significant at 5 % point.

(iii) *Difference in numbers of vegetative and cushion brooms in four Imperial College Selections (ICS 4, 5, 20 and 97)*

The experiments described above also revealed marked differences between the clones (ICS 4, 5, 20 and 97) in number of vegetative and cushion brooms. The results from Exp. (i) are summarized below for vegetative brooms (Table 3) and for cushion brooms (Table 4). Similar results were obtained in Exp. (ii).

It must be left for future experiments to establish whether this result for ICS 4 is only fortuitous, or if there is some peculiarity associated with that clone inducing increase in cushion brooms following applications of Bordeaux mixture. This

TABLE 3. *Numbers of vegetative brooms per tree on four Imperial College Selections, sprayed and unsprayed*

	ICS clone no.			
	4	5	97	20
Treatment: no spray	69.5	35.9	31.1	23.7
Bordeaux mixture	44.6	12.2	5.3	8.0
Mean	57.05	24.05	18.20	15.85
Difference between means	33.00*		5.85	2.35
Critical difference at 5 % point	10.89			

* Difference significant at 5 % point.

TABLE 4. *Numbers of cushion brooms per tree on four Imperial College Selections, sprayed and unsprayed*

	ICS clone no.			
	4	5	97	20
Treatment: No spray	20.4	14.4	11.6	4.6
Bordeaux mixture	64.3	11.7	11.0	3.1
Mean	42.35	13.05	11.30	3.85
Difference between means	29.30*		1.75	7.45
Critical difference at 5 % point	14.65			

* Difference significant at 5 % point.

peculiar result with ICS 4 was obtained in two independent experiments, using different sets of trees. If there is any future investigation of this apparent peculiarity of ICS 4 with respect to applications of Bordeaux mixture, it is suggested that it may be valuable to observe whether there is any effect on the total number of cushions produced in association with the numbers of cushion brooms formed. In the experiments described above, only the numbers of cushion brooms were recorded, consequently, no information is available with regard to the numbers of healthy cushions produced. The differences between ICS 4 and 5 in numbers of vegetative and cushion brooms were apparently greater in these experiments (Tables 3 and 4) than in the observations made by Baker & Crowdy (1942) and by Baker (1943).

(iv) *Fortnightly and weekly applications of fungicides to entire trees*

Only partial control of witches' broom disease was obtained with monthly spray applications. Possibly a better degree of control might be obtained with shorter intervals between the applications, because it is important to ensure that there is an adequate cover of fungicide during the critical period before flushing when infection by spores of *Marasmius perniciosus* is presumed to occur. Unfortunately, it was not convenient to continue the spraying experiments on entire trees at River Estate because many of the trees were becoming too large for satisfactory spray

applications with the equipment available. Further spraying experiments were therefore made on trees of a convenient size at an estate in the Mausica area to observe whether witches' broom disease could be controlled by fortnightly or weekly spray applications. In searching for an effective readily prepared spray, a proprietary fungicide (Perenox (cuprous oxide)) was used in a preliminary experiment (*iva*). In the experiments described below (*iva* and *b*), relatively few brooms were observed, the majority being vegetative brooms. Therefore, vegetative and cushion brooms were not recorded separately in these experiments at Mausica Estate.

Experiment iva. The 5-year-old trees used in this experiment had been vegetatively propagated from a selection referred to as 'River Red' (a type with red pods planted at River Estate). The sprays were applied at intervals of 2 weeks for a period of 3 months, beginning on 19 May 1945 and ceasing on 18 August 1945. There were six plots, each of four trees, providing duplications of the three treatments, which were as follows:

Bordeaux mixture (1%) applied fortnightly
Perenox (0.2%) applied fortnightly
No spray

Results are assembled in Table 5.

TABLE 5. *Mean number of brooms per tree in an experiment comparing Bordeaux mixture (1%) and Perenox (0.2%), applied fortnightly*

No. brooms	Treatment		
	Bordeaux	Perenox	No spray
	0.13	0.88	0.88

From the results obtained in Exp. *iva*, it did not seem worth continuing with Perenox, therefore Bordeaux mixture alone was used in Exp. *ivb*.

Experiment ivb. In this experiment there was a comparison between weekly and fortnightly applications of 1% Bordeaux mixture. The trees were similar to those used in Exp. *iva*. This experiment was maintained for a period of 16 months from 27 August 1945 to 28 December 1946. The treatment effect was not significant in this experiment.

TABLE 6. *Mean number of brooms per tree in an experiment comparing the effects of weekly and fortnightly applications of Bordeaux mixture*

No. brooms	Interval between applications		
	1 week	2 weeks	No spray
	0.83	0.17	1.67

Relatively little witches' broom* disease was recorded during the experiment, the number of brooms averaging less than one per tree. Nevertheless, Exp. ivb demonstrated that regular spraying, as often as once a week for a period of 16 months, does not prevent broom development. Spraying began on 25 August, and the removal and recording of brooms and other diseased shoots was carried out at weekly intervals from then onwards, but any disease which developed in the period 25 August 1945 to 22 September 1945 was ignored in compiling the results. In the plots sprayed every week, the first broom was recorded on 3 November, at which time there had been nine spray applications. The other brooms in these plots were recorded after periods of from 3 to 8 months from the first spraying. In the plots sprayed fortnightly, only two brooms were recorded, the first being on 22 December 1945, when there had been nine spray applications, the other on 30 March 1946, when there had been sixteen spray applications. In view of there having been periods of 3 months or longer from the time when spraying began, it is very unlikely that the brooms which developed were due to infection having taken place before spraying started. Baker & Crowdy (1943, 1944) state that there is an incubation period of 3 weeks to 3 months between infection and the development of a fan broom. It must be concluded, therefore, that the brooms which occurred in the sprayed plots were due to infection which took place in spite of the spray applications. No explanation can be offered for the apparent anomaly that there were more brooms (0.83) recorded for the weekly spraying as compared with the fortnightly spraying (0.17). In spite of the experimental trees having been produced by vegetative propagation, there was considerable variation amongst them in disease incidence. Consequently, because the plots contained only four trees, it was possible for the data from exceptional trees to bias the results.

Spray application to cropping regions of trees

Monthly applications of Bordeaux mixture

This experiment was carried out at River Estate in a field planted in 1933. The experimental area comprised five blocks, each block being the seedling progeny from one of five high-yielding parent trees. Each block contained forty-eight trees. Spray applications began in May 1940, and ended in February 1942. During this period spray was applied every month, excepting the two months of January and February 1941, when no spraying was done. Bordeaux mixture (1%) was applied to the cropping regions of the tree, including the main trunk, branches and all their ramifications which were producing flowers. There was no attempt to spray the foliage of the tree. The diseased pods were separated into two classes, the witches' broom pods and the other diseased pods which were mostly black-pod (*Phytophthora*), *Diplodia* pods and wilted pods, as described by Baker & McKee (1943). The possibility that discrepancies may have arisen through wrong classification of the pods will be considered later. Results are listed in Table 7.

The percentage of witches' broom pods on the sprayed trees was 3.2%, as

TABLE 7. *Mean number of pods per tree in an experiment comparing monthly application of Bordeaux mixture with no treatment*

	Treatment		Difference	Critical difference at 5 % point
	No spray	Bordeaux		
Pod classification: (a) Witches' broom pods	1.34	0.38	0.96*	0.48
(b) Other diseased pods	2.84	1.52	1.32	—
(c) Total diseased pods	4.18	1.90	2.28*	1.26
(d) Total pods	12.82	10.26	2.56	—

* Difference significant at 5 % point.

compared with 10.3 % for the trees which were not sprayed. The latter value for untreated trees was lower than might have been expected for this experiment, which was carried out on an area of River Estate where severe infection had occurred previously. The writer reported (1943) a pod loss of 39.5 % from witches' broom disease for parts of River Estate in the period 1941/2. The percentage of total diseased pods was 32.3 % for the untreated trees, and 17.9 % for the sprayed trees. It is sometimes difficult in the field to distinguish pods affected with witches' broom disease from those suffering from other types of pod disease. Therefore, it is possible that some witches' broom pods may have been incorrectly classified as 'other diseased pods', thereby accounting for the apparently small percentage of pods affected with witches' broom disease in this experiment. Economically, the important feature of the experiment is that there was a significant reduction in numbers of total diseased pods (witches' broom pods plus other diseased pods) which can be attributed to the monthly applications of 1 % Bordeaux mixture to the cropping regions of the trees. The possibilities in this connexion have been examined further by determining the regression coefficients for total pods (X) on total diseased pods (Y) in 111 untreated trees (b_1), and for 107 sprayed trees (b_2) respectively. These regression coefficients are, $b_1 = +0.282$, $b_2 = +0.190$, the difference between them (0.092 ± 0.029) being highly significant.

PINK DISEASE (*CORTICIUM SALMONICOLOR*)

Rorer (1916) reported the occurrence of pink disease (*Corticium salmonicolor*) in Trinidad and suggested that Bordeaux mixture might be used to control it. Observations at Mausica Estate showed that cacao trees had this disease, which affected both small and large branches. Accordingly, observations were made on the incidence of pink disease on sprayed and on untreated trees.

RESULTS

Fortnightly applications of spray to entire trees

The three treatments were: Bordeaux mixture 1 %; Perenox 0.2 %; no spray.

A description of the trees used for this experiment is given under witches' broom disease (p. 366). The results obtained are summarized in Table 8.

The table shows that fortnightly Bordeaux applications controlled pink disease. Perenox was relatively ineffective. Observations were continued to determine residual spray effects against infection by *C. salmonicolor*. On the Bordeaux-sprayed plots the first case of pink disease was noticed on 2 November 1946, 14 months from the end of spraying. In the Perenox-sprayed plots, pink disease appeared on 19 January 1946 and on 17 November 1945, 5 and 3 months, respectively, from the cessation of spraying.

TABLE 8. *Mean number of affected shoots per tree in an experiment comparing the effect of two different spray treatments on pink disease*

Treatment		
Bordeaux	Perenox	No spray
0.00	0.25	1.38

Weekly and fortnightly applications of Bordeaux mixture to entire trees

The primary object of this experiment was to determine the effect on witches' broom disease of a reduction in the length of the interval between spray applications from 2 weeks to 1 week; incidental observations were made on the occurrence of pink disease. Further details have been given under witches' broom disease (Exp. ivb). The results are summarized in Table 9.

TABLE 9. *Mean number of affected shoots per tree in an experiment comparing weekly and fortnightly applications of Bordeaux mixture*

Interval between applications		
1 week	2 weeks	No spray
0.00	0.00	3.25

The table shows that complete control of pink disease was obtained with both weekly and fortnightly applications of Bordeaux mixture, thereby confirming similar results obtained when fortnightly applications only were given.

BLACK-POD DISEASE (*PHYTOPHTHORA PALMIVORA*)

INTRODUCTION

A rational approach to the problem of controlling black-pod disease may be said to have begun with Rorer's studies (1910) on the life history of the fungus. A species of *Phytophthora* had been earlier recognized as the cause of this pod rot of cacao by Massee (1899), but Busse (1905) had carried out inoculation experiments and had made certain tentative recommendations as regards control measures. Rorer (1910) advocated spraying and considered that at least four applications should be given in Trinidad. Laycock began spraying experiments in Nigeria which were continued by West (1936), who found that spraying three times a year was

uneconomic. More recently, Owen (1951) carried out experiments in the Gold Coast which included weekly applications of both Bordeaux mixture and Perenox, and commented that such frequent applications could never be economic. Perenox was found to be effective but less so than Bordeaux mixture.

The experiments described in this paper were made to obtain more precise information on the effects of different frequencies of fungicide application. The possibility of using Perenox in Nigeria for controlling black-pod disease was a subsidiary object of investigation.

EXPERIMENTAL DETAILS

Location

All the experiments were carried out at Owena, a cacao experiment station of the Department of Agriculture in Ondo Province. The position of this Station is shown elsewhere (Thorold, 1952), together with certain details of its climate and black-pod incidence.

Type of cacao

Seedling trees of West African Amelonado type were used. Many of the trees at Owena were impaired through severe capsid attack (Crowdy, 1947), leaving only a limited number for the experiments. Accordingly, plots were selected containing the requisite numbers of bearing trees; subsequently, the treatments were allocated to the plots at random.

Spraying equipment

'Four Oaks', Kent pattern (4 gallon) spray-pumps were used for the application of the fungicides. It was not possible for the spray to reach all the pods satisfactorily on account of the height of some of the trees. No attempt was made to treat parts of the tree other than the developing fruits.

Fungicides

'Carbide Bordeaux' (McDonald, 1937) was used because of lack of lime. It was customary in Kenya to refer to this fungicide as carbide Bordeaux, so the term is retained, in spite of its being a misnomer. Copper sulphate was used at 1% in water, and the appropriate amount of solid calcium carbide was added immediately before application of the fungicide (1 lb. CuSO_4 , 6 oz. CaC_2 , in 10 gallons water). The evolution of acetylene gas obviates the necessity for stirring the mixture during preparation, and the black spray deposit is conspicuous and tenacious. Perenox was used at a concentration of 0.1% in water, and applied to the pods in a similar manner to the carbide Bordeaux.

Treatment and lay-out

Experiment A. 1% carbide Bordeaux applied at intervals of 1, 2, 3 and 4 weeks respectively was contrasted with no spray. The treatments began in April 1950 and ceased in March 1952. Every plot contained six trees and there were six replications

of each of the five treatments (0, 1, 2, 3, 4-weekly applications of 1% carbide Bordeaux).

Experiment B. 1% carbide Bordeaux and 0.1% Perenox were applied at monthly intervals and compared with no spray. The treatments began in May 1950 and ceased in March 1952. Single trees served as plots, and fourteen trees were allocated to each of the three treatments (1% carbide Bordeaux, 0.1% Perenox, no spray).

Crop and disease records

Healthy and diseased pods were recorded individually. Healthy pods were picked when ripe, but affected pods were picked every week when black-pod symptoms appeared. All pods were removed from the experimental area for opening. The contents of every pod were weighed when the wet beans could be considered as likely to be marketable after fermentation and drying. Some of the affected pods contained marketable cocoa, but in those that were immature the beans were discarded without weighing. As there is generally an average loss of 58% in weight when wet beans are fermented and subsequently dried for export, the recorded weights were converted to an equivalent weight of dry cocoa by multiplying by 0.42.

It is the local custom to consider the cocoa-crop season as extending from 1 April in one year until the following 31 March. This custom is followed in presenting the results from the experiments. Data were obtained in two crop seasons, which are referred to below as 1950/1 and 1951/2 respectively.

RESULTS

Experiment A: Effect of different intervals between applications of 1% carbide Bordeaux

Number of black-pods

Table 10 shows the mean number of black-pods per tree in 1950/1 and 1951/2 seasons respectively.

TABLE 10. *Mean number of black-pods per tree*

	Interval between spray applications (weeks)					Significance level of treatment effect (<i>P</i>)	Critical difference
	0	1	2	3	4		
1950/1	9.5	0.9	0.3	0.6	0.7	< 0.01	2.5
1951/2	6.3	0.6	0.2	0.2	0.4	< 0.01	3.1

Number of total (healthy plus diseased) pods

Table 11 shows the mean number of total pods per tree in 1950/1 and 1951/2 seasons respectively.

It seemed probable from the second season's results that the significant treatment effect in the first season was fortuitous. It was unreasonable to suppose that either fortnightly or monthly applications had really affected the number of pods adversely,

in view of the observation that neither weekly (14.3) nor 3-weekly (15.7) applications differed significantly from the control trees (16.3).

TABLE 11. *Mean number of total (healthy plus diseased) pods per tree*

	Interval between spray applications (weeks)					Significance level of treatment effect (<i>P</i>)	Critical difference
	0	1	2	3	4		
1950/1	16.3	14.3	8.4	15.7	7.4	<0.01	5.2
1951/2	10.8	10.8	7.2	6.8	6.1	>0.5	—

Weight of dry cocoa (wet cocoa weight $\times 0.42$)

Table 12 shows the mean weight of dry cocoa per tree in 1950/1 and 1951/2 seasons respectively.

TABLE 12. *Mean weight of dry cocoa per tree (oz.)*

	Interval between spray applications (weeks)					Significance level of treatment effect (<i>P</i>)	Critical difference
	0	1	2	3	4		
1950/1	10.8	20.8	11.0	24.1	12.2	<0.01	7.7
1951/2	4.9	12.7	8.3	8.8	6.8	<0.05	4.7

Experiment B: Comparison of 1% carbide Bordeaux and 0.1% Perenox, applied at monthly (4-week) intervals

Number of black-pods

Table 13 shows the number of black-pods recorded for trees receiving carbide Bordeaux and Perenox treatments in 1950/1 and 1951/2 respectively.

TABLE 13. *Mean number of black-pods per tree*

Treatment	Control	Carbide Bordeaux	Perenox	Significance level of treatment effect (<i>P</i>)	Critical difference
1950/1	20.9	10.1	11.1	<0.05	8.9
1951/2	13.7	9.6	8.3	>0.05	—

Number of total (healthy plus diseased) pods

Table 14 shows the number of total pods recorded for trees receiving 1% carbide Bordeaux and 0.1% Perenox treatments in 1950/1 and 1951/2 seasons respectively.

TABLE 14. *Mean number of total (healthy plus diseased) pods per tree*

Treatment	Control	Carbide Bordeaux	Perenox	Significance level of treatment effect (<i>P</i>)	Critical difference
1950/1	27.9	32.1	26.1	>0.05	—
1951/2	19.4	26.2	17.1	>0.05	—

The treatment effect on total pods was not significant in either season.

Weight of dry cocoa (wet cocoa weight $\times 0.42$).

Table 15 shows the mean weight of dry cocoa per tree in 1950/1 and 1951/2 seasons respectively.

TABLE 15. Mean weight of dry cocoa per tree (oz.)

Treatment	Control	Carbide Bordeaux	Perenox	Significance level of treatment effect (P)	Critical difference
1950/1	12.8	43.6	23.6	<0.01	14.6
1951/2	5.8	16.4	10.8	<0.05	8.3

Combination of data from experiments A and B

Differential effects of fungicide applications with respect to position of pods on the tree

It is explained above that the spray equipment was unsatisfactory, in that it did not treat the 'high' pods adequately, i.e. those more than 7 ft. above the ground. All the pods picked in Exps. A and B during 1951/2 were classified as 'high' or 'low' and also as healthy or diseased. The results are summarized in Tables 16 and 17.

TABLE 16. Numbers of healthy pods and of black-pods recorded on sprayed trees in 1951/2

Position	High	Low	Total
Healthy pods	381	898	1279
Black-pods	222	74	296
Total	603	972	1575

$$\chi^2 = 207.945, \text{ significant } (P < 0.01).$$

TABLE 17. Numbers of healthy pods and of black-pods recorded on untreated trees in 1951/2

Position	High	Low	Total
Healthy pods	98	158	256
Black-pods	192	238	430
Total	290	396	686

$$\chi^2 = 2.668, \text{ not significant } (P > 0.1).$$

Relationship between number of black-pods and number of total pods

The writer has shown (Thorold, 1952) that the number of black-pods per tree (Y) tends to vary directly with the number of total pods per tree (X). This relationship has been examined for the sprayed and the untreated trees respectively in 1950/1. The results are summarized in Table 18.

The difference between the regression coefficients was greater in Nigeria (0.408) than in Trinidad (0.092), indicating that there was a smaller increase in pod disease

per unit increase in number of total pods on sprayed trees in Nigeria. It is probable that this apparently superior benefit from spraying in Nigeria may be attributed to the fact that the only pod losses considered were those due to *Phytophthora* infection. In the Trinidad observations, 'total diseased pods' included 'wilted pods', the number of which was presumably not affected by spraying because pod wilt is believed to be a physiological disease.

TABLE 18. *Correlation and regression coefficients for number of total pods per tree (X) and number of black-pods per tree (Y)*

	No. trees	Correlation coefficient (r_{XY})	Regression coefficient (b_{XY})	Difference between regression coefficients
Untreated trees	44	+0.867*	+0.659	
Sprayed trees	145	+0.666*	+0.251	0.408 ± 0.087

* Significant at 1 % level.

Influence of certain uncontrolled factors

Exp. A, which was intended to compare the effectiveness of different frequencies of fungicide applications, has failed to show any consistent differences. In another experiment in the same area, a less satisfactory degree of control was obtained. It is convenient to illustrate these general observations by a summary of the results from the two experiments in terms of black-pod percentages

$$\left(\frac{\text{no. black-pods per tree} \times 100}{\text{no. total pods}} \right)$$

given in Table 19.

TABLE 19. *Number of black-pods expressed as percentages of total pods in two experiments with applications of 1% carbide Bordeaux*

	Interval between applications (weeks)						
	0	1	2	3	4	4	0
	Experiment A					Experiment B	
1950/1	58.4	6.0	3.6	3.7	9.8	31.4	74.0
1951/2	58.5	5.2	2.7	2.9	6.8	36.5	70.6
Two seasons combined	58.5	5.7	3.2	3.5	8.4	33.7	73.2

The table shows two features in particular which require elucidation:

(a) A relatively large black-pod percentage (5.7) for trees receiving weekly (1) applications.

(b) A wide divergence in black-pod incidence in the two experiments after monthly (4) applications (8.4 and 33.7%).

These two inconsistencies ((a) and (b)) will now be considered in detail:

(a) The individual tree records show that one tree in particular has unduly biased the results in the weekly applications of carbide Bordeaux (cf. exp. iv b).

This tree had a larger percentage (57%) of 'high' pods in 1951/2 as compared with that of all the trees in this experiment (35%), which accounts for the apparently less satisfactory level of black-pod control in the case of the weekly treatment (Table 19 (1)).

(b) It is evident on comparing the data for the untreated trees (c) in the two experiments, that the incidence of black-pod was greater in Exp. B (73.2%) than in Exp. A (58.5%). The trees used in Exp. B were generally taller than those in Exp. A. This difference in size of trees is probably connected with a wider spacing (12 × 12 ft.) in Exp. B, as compared with 8 × 8 ft. in Exp. A. This difference in size of tree was associated with a preponderance of 'high' pods in the trees in Exp. B (52%), as compared with 37% of 'high' pods in Exp. A. It seems probable that black-pod disease incidence in Exp. B may also have been enhanced as compared with Exp. A through localized differences in intensity of infection. The site of Exp. B happened to be near the site of another experiment where black-pods were allowed to remain on the trees. On the other hand, the site of Exp. A was near the site of another experiment where trees were inspected and black-pods removed on alternate days. Circumstantial evidence in support of such differential effects has been obtained in Fernando Po, where Bordeaux mixture is almost universally used on plantations for controlling black-pod disease (Nosti, 1947). Consequently, there are relatively few sources of infection, so that the minimum number of applications per season is two and the maximum number is four. In the Ureka area, where spraying has been neglected, much black-pod was seen. The Ureka district is an area of very high rainfall (Nosti, 1942) similar to the Debundscha area in the Cameroons (Thorold, 1952). There is reason to believe that conditions for potential black-pod incidence are generally comparable in Fernando Po with parts of Nigeria, including Owena. Longer intervals between applications are found to be satisfactory in Fernando Po, as compared with experience from small-scale experiments at Owena. The use of relatively few applications of fungicide for the control of black-pod is probably dependent on the extent to which control measures are practised, thereby influencing the general level of *Phytophthora* infection.

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FACTORS AFFECTING THE PRODUCTION OF LOCAL LESIONS BY PLANT VIRUSES

I. THE EFFECT OF TIME OF DAY OF INOCULATION

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(With 2 Text-figures)

Under uncontrolled glasshouse conditions the number of local lesions produced by a tobacco necrosis virus in beans depended on time of day at which plants were inoculated. Inoculations in the afternoon produced most lesions and inoculations near the end of the night produced least. Tobacco mosaic virus in *Nicotiana glutinosa*, and lucerne mosaic and turnip mosaic viruses in tobacco showed similar but smaller effects. The variations could affect the results of quantitative experiments with local-lesion counts.

Since Holmes (1929) showed that *Nicotiana glutinosa* produced countable local lesions when inoculated with tobacco mosaic virus, local-lesion counts have been widely used to estimate the relative amounts of infectious virus in different preparations. Present knowledge of the factors affecting local-lesion production has been summarized and critically discussed by Bawden (1950). Results of some unpublished experiments on the chemotherapy of plant viruses suggested that the number of local lesions produced was varying systematically with the time of day at which plants were inoculated, and this was confirmed by experiments here described.

MATERIALS AND METHODS

Except where noted otherwise, plants were grown singly in 4 in. pots. Plants used in any experiment were uniform in appearance. Experiments were made between March and May in a glasshouse with a light whitewash covering. Bean plants (*Phaseolus vulgaris* var. Sydney Wonder) were used when the primary leaves were not quite fully expanded, and the main shoot was removed before inoculation. *Nicotiana glutinosa* plants were used when they had seven leaves suitable for inoculation. Growing points were removed 2 days before inoculation. Tobacco plants (*N. tabacum* var. White Burley) were used when three or four leaves were well expanded but before the stems became elongated. Leaves were lightly dusted with fine carborundum powder before inoculation, and were not washed afterwards.

The tobacco necrosis virus came originally from lettuce roots (Fry, 1952); inoculum for these experiments was sap from infected bean leaves. The tobacco mosaic virus came originally from smoking tobacco, and inoculum for these

experiments was freshly expressed tobacco leaf sap. The lucerne mosaic virus came originally from clover (Fry, 1953) and inoculum was sap from systemically infected *N. glutinosa* plants. Turnip mosaic virus inoculum was sap from infected turnip leaves. Preliminary dilution tests were carried out to determine a suitable strength of inoculum for each experiment. One bulk dilution (using distilled water) was then made, and aliquots taken at each time of inoculation. Inoculum containing tobacco necrosis and tobacco mosaic viruses was kept at room temperature. Inoculum containing lucerne mosaic and turnip mosaic viruses was stored at $+4^{\circ}\text{C}$.

Uniform plants were arranged in groups, randomized within groups, numbered, and then rearranged so that the no. 1 plants for all times were in one tray, no. 2 in the next, and so on. Half of every leaf was inoculated at base time (8 a.m. or 10 a.m.). The actual time taken to do this inoculation (1–2 hr.) was spread approximately evenly around the nominal base time. All no. 1 plants were inoculated first, followed by number 2, etc. In this way any effect of changing susceptibility during base time inoculations could be largely equalized between groups.

The left hand side of one bean leaf and the right-hand side of the other were inoculated at base time, the remaining half-leaves being inoculated at one of the test times. The leaves on the odd-numbered plants of *N. glutinosa* and *N. tabacum* in each group were inoculated at base time on the left-hand side and the leaves on the even-numbered plants on the right-hand side.

At test times plants in each group were inoculated in order from no. 1 to 8. Test-time inoculations took 5–10 min. All inoculations in each experiment were made by one operator, except in exp. 3 where half the base-time inoculations were done by each of two operators.

For graphical presentation of results $\log R$ is plotted against time of inoculation:

$$\log R = \frac{\sum [\log_{10}(x+1) - \log_{10}(b+1)]}{n},$$

where b = number of local lesions on base-time half-leaf,

x = number of local lesions on test-time half-leaf,

n = number of half-leaves.

To determine the significance of differences between values of $\log R$ the t test was used.

For sugar estimations, leaves were detached, weighed and placed immediately in 87% ethanol (10 ml. per g. fresh weight of leaf) and boiled for 3 min. Alcohol was then removed from the extract by boiling, with the addition at intervals of small amounts of water. The green precipitate was centrifuged off and the extract brought (in an oven at 80°C .) to 0.2 ml. for each g. of fresh leaf. For chromatography 0.006 ml. of each extract was placed on the paper (Whatman's no. 1) and run for 20 hr. in butanol-acetic acid-water (Partridge, 1948). Sugar spots were located by spraying with a benzidine trichloroacetic acid mixture (Bacon & Edelman, 1951).

EXPERIMENTAL

Tobacco necrosis virus in beans

Experiment 1. A different group of sixteen plants was inoculated every 2 hr. over a 24 hr. period, and one 48 hr. after base time. The mean number of local lesions per half leaf for base time (8 a.m.) for all groups was 76. Results are summarized in Fig. 1*a*.

Experiment 2. In addition to the test times used in Exp. 1, one set of plants was inoculated 24 hr. before base time (8 a.m.). The mean number of local lesions for the base time half-leaves was 28.1. Results are given in Fig. 1*b*.

The results for both experiments show the same general trends. The numbers of local lesions increased during the morning, reached a maximum in the afternoon, and fell to a minimum at 6 a.m. in Exp. 1, and 4 a.m. in Exp. 2. In Exp. 2 there was a significant rise between 4 a.m. and 6 a.m. At 6 a.m. there was no detectable rise in light intensity, light being first registered on an exposure meter 40 min. later.

Plants from the same batch of beans were used to obtain an indication of the variation in content of sugars in the leaves over the time covered by Exp. 2. Four bean plants, two per 4 in. pot, were used for each sampling. Samplings were made in duplicate at each time plants were inoculated. For chromatography the extracts were run on two sheets, one sample for each time being on each sheet. Two spots appeared with the same R.F. values as sucrose and glucose. Although this is no proof of identity, it is almost certain that these are the sugars involved since they are the ones normally found predominantly in leaves. The sugar rose to a maximum around 4 p.m. and fell during the night. This trend was more definite with glucose than with sucrose.

Tobacco mosaic virus in Nicotiana glutinosa

Experiment 3. This experiment was made on the same day as Exp. 2 under similar conditions. Seven leaves on each of eight plants were inoculated for each test time. Nominal base time was 10 a.m. On each plant leaves of different age were inoculated in random order both at base time and test times. Mean number of local lesions for the base-time half-leaves was 39.6. The values of log *R* for each leaf are plotted in Fig. 2.

The biggest rise and fall occurred in the youngest leaf, while in leaves 2 and 5 there was no significant rise during the day. For all leaves combined (Fig. 1*c*) there is a significant rise from 12 a.m. to a maximum at 6 p.m., followed by a fall during the night.

Incidentally it was found that there were significant differences among the standard errors of log *R* for the different leaves. There was a trend of decreasing standard error from leaf 1 to leaf 4, followed by an increase for the top three leaves.

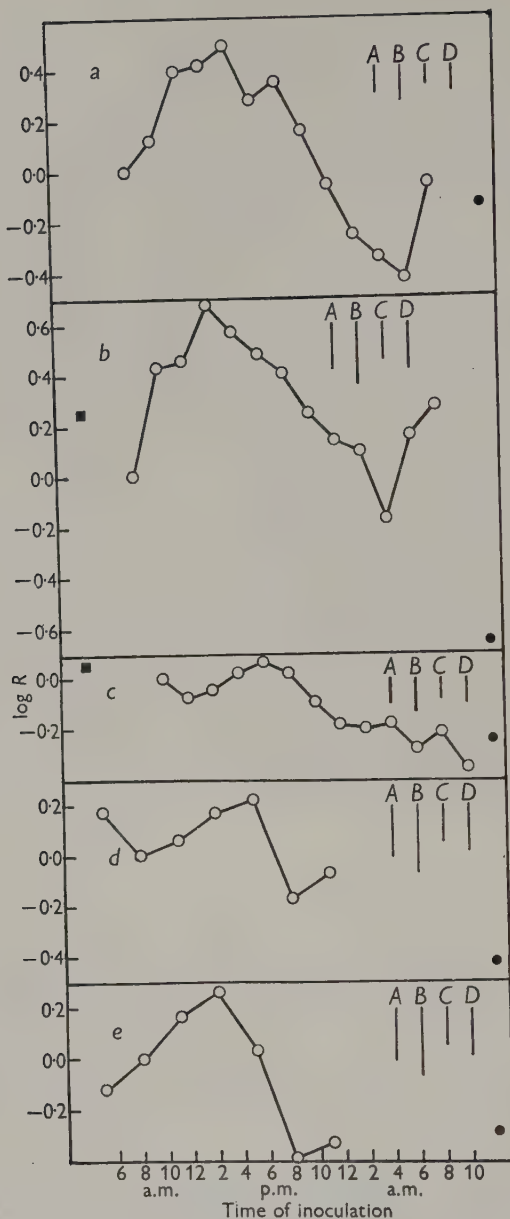


Fig. 1. Effect of time of day of inoculation on number of local lesions. *a*, tobacco necrosis virus in beans; *b*, tobacco necrosis virus in beans; *c*, tobacco mosaic virus in *N. glutinosa*; *d*, lucerne mosaic virus in tobacco; *e*, turnip mosaic virus in tobacco. Lines represent differences required for significance as follows: *A*, between any two points at 5%; *B*, between any two points at 1%; *C*, between any point and base time at 5%; *D*, between any point and base time at 1%. Solid squares represent points 24 hr. before base time; solid circles, points 48 hr. after base time.

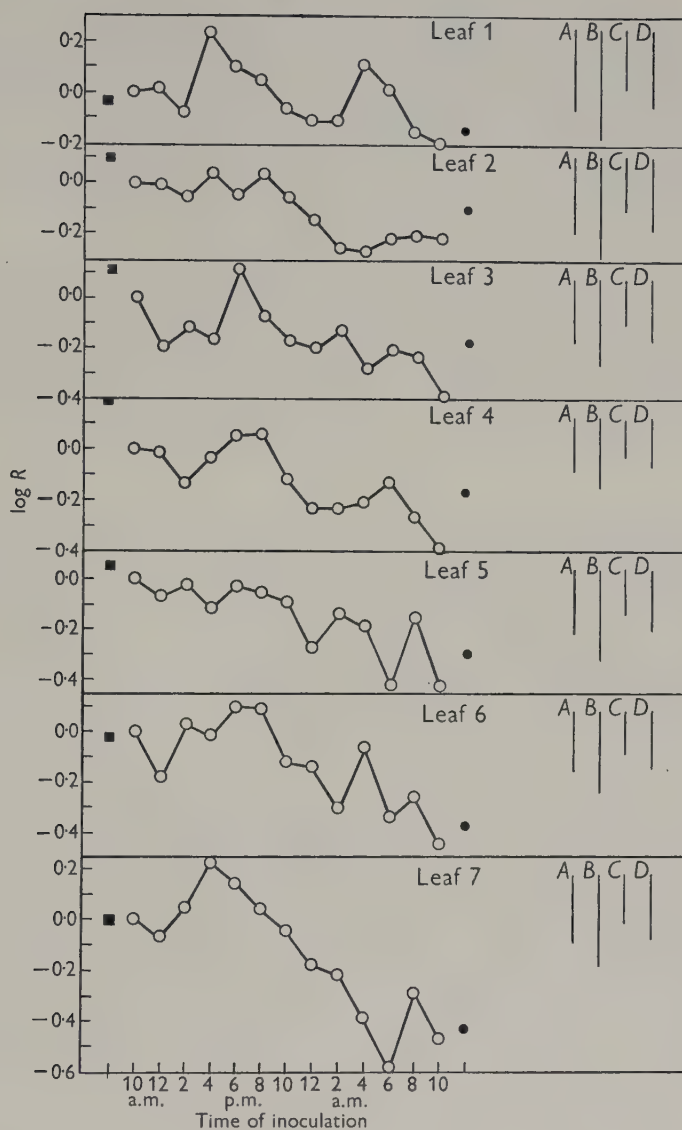


Fig. 2. Effect of time of day of inoculation on number of local lesions produced by tobacco mosaic virus in *N. glutinosa*. Leaves numbered in order from leaf 1 (oldest) to leaf 7 (youngest) leaf. Lines represent differences required for significance as follows: A, between any two points at 5%; B, between any two points at 1%; C, between any point and base time at 5%; D, between any point and base time at 1%.

Lucerne mosaic virus in tobacco

Experiment 4. Three leaves on each of ten tobacco plants were inoculated at each test time. The lowest leaf was discarded, as powdery mildew (*Erysiphe*) infection made accurate lesion counts impossible. The mean number of lesions per half-leaf for the base time (8 a.m.) was 9.8. A combined curve for both leaves is plotted in Fig. 1*d*. There was a small rise during the day to 5 p.m. followed by a steep fall to 8 p.m.

Turnip mosaic virus in tobacco

Experiment 5. This experiment was done on the same day in the same glasshouse as Exp. 4, using the same numbers of leaves, plants and times, but slightly older plants. The mean number of lesions per half-leaf for base time (9 a.m.) was 12.8. The combined results for the three leaves are plotted in Fig. 1*e*. Again there was a rise and fall during the day, the maximum being at 2 p.m.

DISCUSSION

The four host-virus combinations behaved similarly in that the number of local lesions produced rose to a maximum during the day and fell to a minimum during the night, and it seems probable that such differences in susceptibility during a period of 24 hr. occur generally with plant viruses. The size of the differences will, no doubt, be correlated both with daily fluctuations in light and temperature and with any of the numerous activities of the plant which show a daily periodicity. The time of inoculation at which most local lesions were produced corresponded approximately to the maximum accumulation of sugars produced by photosynthesis; this renders unlikely a suggestion made by Bawden & Roberts (1948) that, during photosynthesis, products accumulate which tend to inhibit local lesion production.

In few of the experiments in which local lesions have been used to estimate infectious virus has the possibility been considered that time of day of inoculation could affect the number of local lesions. In an experiment not designed to eliminate the effect of time of day, it would either increase the 'error' variance or cause a systematic bias in the results. When two or more virus preparations are compared each preparation is usually inoculated at several dilutions in some suitable experimental design, usually employing half-leaves as the unit. The most convenient method, and therefore probably the one most commonly used, is to inoculate one preparation at a time, beginning with the highest dilution and working in order to the lowest. If the number of plants, or the diligence of the operator, is such that the inoculations take more than 1-2 hr. to complete, the susceptibility of the plants could change enough both to distort the slope of the dilution curves and to affect the relative numbers of local lesions produced by the preparations under test. If, alternatively, one half-leaf is inoculated in turn with each dilution of each preparation, the effect of changing susceptibility will tend to decrease the statistical

significance of the results obtained. When the time taken for inoculations is 1 hr. or less, changing susceptibility probably has no significant effect on results. Where the time for inoculation is unavoidably long, the effect could be greatly reduced by an experimental design in which the effect is taken out in block differences. For example, the method using a standard preparation inoculated on half of every leaf will be useful, as each sample can be compared with the standard by inoculation over a short period. The need for this type of design is illustrated by data obtained by Mr P. R. Fry of the Plant Diseases Division with local lesions produced by turnip mosaic virus in tobacco. He was comparing seven samples. The inoculations were made between 2.25 p.m. and 4.40 p.m., two leaves on each of twelve plants being used for each comparison. The total number of local lesions obtained for each set of standard half leaves, in order of time of inoculation, was as follows: 1572, 962, 762, 909, 594, 374 and 124. Further work on the effects of light and temperature may make it possible to define conditions for raising plants which will not show a continuous change in susceptibility.

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THE TRANSMISSION OF PLANT VIRUSES BY BITING INSECTS, WITH PARTICULAR REFERENCE TO COWPEA MOSAIC

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Experiments on the virus-vector relationship of the Trinidad cowpea mosaic virus, transmitted by *Ceratoma ruficornis*, gave the following results: ability to infect decreased with increasing time after ceasing to feed on infected plants, but vectors remained infective for 14 days (much longer than the longevity *in vitro* of the virus at glasshouse shade temperatures of 23–31° C.); the beetles transmitted more consistently after longer feeding on infected plants, though feeds of under 5 min. made them efficient vectors; the proportion of plants infected increased with the amount of feeding damage on them; fasting the vectors before feeding on infected plants increased voracity but had no effect on their ability to transmit; beetles became infective immediately after feeding on infected plants. Cowpeas were infected by inoculation with macerated infective vectors or with juice regurgitated by vectors. There is no evidence that aphids or other sucking insects can transmit the virus. It seems similar to squash mosaic and turnip yellow mosaic, for vectors of all three viruses probably transmit by regurgitating infective juice during feeding.

INTRODUCTION

In contrast to the many insects with sucking mouthparts that transmit plant viruses, there are few reliable records of biting insects acting as vectors. Doolittle (1920) reported the transmission of cucumber mosaic in the U.S.A. by *Diabrotica* spp. and *Aphis gossypii* Glov.; but his claim concerning beetles is unconfirmed and aphids are now considered the usual vectors. C. E. Smith (1924) found the bean leaf-beetle (*Ceratoma trifurcata* Forst.) was an efficient vector of a cowpea mosaic in the U.S.A. and transmitted the virus after only 5 min. feeding on infected plants. Grasshoppers (*Melanoplus* spp.), flea-beetles (*Epitrix cucumeris* Harris and *Systema taeniata* Say.), the larva of the Colorado beetle (*Leptinotarsa decemlineata* Say.) and a leaf beetle (*Disonycha triangularis* Say.) can transmit potato spindle-tuber, and all but the last are vectors of potato unmottled curly dwarf, which is probably due to a strain of the same virus (Goss, 1931); both diseases also have various sucking vectors, mainly aphids. Bonde & Merriam (1951) have shown that these vectors are sometimes not very effective in transmitting spindle-tuber, which is more readily spread mechanically during storage, handling and planting tubers. Larson & Walker (1939) transmitted a cabbage mosaic in Wisconsin by larvae of the Small White butterfly (*Pieris rapae* L.).

Freitag (1941a) reported transmitting four cucurbit viruses in California by *Diabrotica* spp. and aphids, as follows: squash mosaic readily by *D. trivittata* Mann.

(its most efficient vector) and *D. soror* Lec., but rarely by aphid species; cucurbit ring mosaic by both beetles, but not by aphids (seven species tested); wild cucumber (*Echinocystis fabacea* Naud.) mosaic by *Diabrotica trivittata*, but not by aphids (nine species tested); western cucumber mosaic once only by *D. trivittata*, but readily by ten aphid species. Single beetles of *D. trivittata* and *D. soror*, after feeding on a source of squash mosaic, infected nineteen out of thirty and two out of twenty-five squash plants respectively. The former, after several days on diseased plants, remained infective for 10 days when transferred daily to successive healthy ones (Freitag, 1941*b*). Freitag (1950, *in litt.*) has since found that cucumber beetles can retain squash mosaic for 20 days.

Markham & Smith (1949) showed that larvae of the mustard beetle (*Phaedon cochleariae* Fabr.) could still infect plants with turnip yellow mosaic 3 days after they had fed on infected plants for only 1 min. When individuals were fed on infected plants for longer periods, and transferred serially to new healthy plants on 4 successive days, half their number infected the fourth plant. Smith (1951) has since reported retention of infectivity by this insect for a week. In similar experiments with adult flea-beetles (*Phyllotreta* spp.), which are largely responsible for natural spread, and the short-horn grasshopper (*Chorthippus bicolor* Charp.), infectivity was retained for at least 3 days (Markham & Smith, 1949). The earwig (*Forficula auricularia* Linn.) and long-horn grasshopper (*Leptophyes punctatissima* Bosc.) were also shown to be vectors. Attempts to transmit turnip yellow mosaic by sucking insects (aphids and capsids) or caterpillars (*Pieris* spp.) were unsuccessful.

Dale (1949) showed that *Ceratoma ruficornis* (Oliv.) can transmit cowpea mosaic in Trinidad. The efficiency of this abundant and active vector fully explains the prevalence of the disease. Although other biting insects may be able to transmit it, this has not been demonstrated; such possible vectors are much less numerous on cowpea and could be of secondary importance only. There is no evidence of transmission by sucking insects. When *Aphis craccivora* Koch (= *A. medicaginis* Koch), already tested as a vector with negative results (Dale, 1949), *A. gossypii*, and undetermined white-flies, thrips and green jassids were allowed to multiply amongst adjacent uncaged diseased and healthy plants, no new infections occurred.

Walters (1951) found that a grasshopper (*Melanoplus differentialis* Thom.) can transmit potato virus X, tobacco ring-spot and tobacco mosaic, but loses its infectivity in 24 hr. There are previous reports of transmission of the last by aphids (Hoggan, 1931; Gigante, 1938), but none concerning the other two. Experiments on the transmission of tobacco mosaic from diseased to healthy tobacco plants by individual grasshoppers (Walters, 1951) suggested that the number of infective insects is increased by increasing both the infection-feeding time and the test-feeding time; a delay between infection feeding and test feeding reduces the number of infective insects. With no delay, almost 30% of the insects transmitted, whereas with a delay of 12 hr., less than 10% did so. The Trinidad egg-plant mosaic virus, which is transmitted by a flea-beetle (*Epitrix* sp.), also has a short life in the vector,

which becomes non-infective in less than a day. Though this vector is inefficient, its abundance may account for the widespread occurrence of the disease. *Aphis gossypii*, also very common on egg-plants, is not a vector.

EXPERIMENTS WITH COWPEA MOSAIC

The experiments described below elucidate certain features of the virus-vector relationship of cowpea mosaic and allow comparison with other viruses. As it proved impossible to breed sufficient insects for these trials, they were collected (except where otherwise stated) from crops of *Phaseolus vulgaris* L., which is not susceptible to cowpea mosaic, and caged until needed with healthy cowpeas. These were observed for mosaic, but it never developed. Single beetles were used in all experiments. The young cowpeas used as sources of virus or as healthy test plants were as uniform as possible.

TABLE 1. *Effect of source-access period on vector efficiency and retention of infectivity*

		Percentage transmissions for different periods on infected plants			
		Under	3 hr.	24 hr.	Prolonged
Serial transfers: 3 hr. per day on successive healthy plants, following initial feeding on infected ones	Days	5 min.			
	0	75	51	79	86
	1	53	60	74	93
	2	52	50	78	82
	3	29	40	48	78
	4	35	42	39	63
	5	26	22	48	42
	6	14	14	31	30
	7	19	13	20	17
	8	7	8	15	9
	9	2	0	2	6
	10	0	2	8	6
	11	0	0	4	3
	12	0	0	0	0
	13	0	0	0	0
	14	0	0	0*	2*

* Continued up to 16 days without additional transmissions.

Table 1 summarizes the results of serial experiments in which beetles were caged for varying periods on diseased cowpeas, transferred directly to healthy plants for 3 hr. and thereafter to successive healthy plants for the same period daily. Before caging on the source of infection, the vectors were starved for 24 hr. to increase their voracity. This procedure was varied for trials involving prolonged source access, beetles being collected from mosaic-infected cowpea crops and kept on diseased plants for a week or more before direct transference to healthy test plants. For each source-access period at least seven separate trials were conducted, like the one represented in Table 2. Initially there were usually eight beetles in each, and though some died, every percentage in Table 1 is based on over forty test plants.

Vectors used in the 5 min. series were timed while feeding on the source, and only those that did so for at least 2 min. (continuously or intermittently) were used. It was impracticable to keep similar records for the other series, but beetles that caused no leaf damage were rejected. The remainder varied greatly in the time spent actually feeding. In general, leaf damage (and therefore source-feeding time) was proportional to the period of access to the source plants.

TABLE 2. *Result of a single trial: under 5 min. feeding on infected plants; 3 hr. on test plants*

Days	Individual beetles							
	A	B	C	D	E	F	G	H
0	+	—	+	+	—	+	+	—
1	—	—	+	+	—	—	—	+
2	—	+	+	—	Dead	+	—	+
3	—	—	+	—	.	—	—	—
4	—	—	—	Dead	.	+	—	+
5	—	—	—	.	.	—	—	—
6	—	—	—	.	.	—	—	—
7	—	—	—	.	.	—	—	+
8	—	—	—	.	.	—	—	+

Serial transfers as in Table 1

Continued without additional transmissions for a further 6 days.

+ Infected plant. — Uninfected plant.

Efficiency of the vectors decreased with time after source feeding, and infectivity was lost by all within 15 days. The data were subjected to angular transformation (Snedecor, 1946) and treated statistically. A regression analysis showed that linear regression lines appear to fit the data adequately, as the coefficients of linear regression for the four series are significant. For the under 5 min., 3 hr. and 24 hr. periods these do not differ significantly amongst themselves, but those of the first two differ significantly ($P=5\%$) from that of the prolonged period. This implies that the infectivity of beetles given prolonged source access diminished more steeply; as they had higher initial infectivity, however, this treatment provided individuals that remained infective for the longest time. Mean values were calculated for each series. These measure the average percentage success. The 5 min. and 3 hr. series are similar, the difference between their means being small and statistically non-significant. The means for the 24 hr. and prolonged series do not differ significantly, but both are significantly different from those for the other two ($P=0.1\%$). The data were also treated collectively by an analysis of variance on the transformed percentages. This confirms that there are significant differences within the regressions and means of the four series.

Thus, vectors having access to infected material for 24 hr. or more transmitted much more efficiently than those permitted shorter access periods. Absence of a significant difference between the prolonged and 24 hr. series probably indicates that after 24 hr. source access, vectors approach the limit of their virus-retaining

capacity. Lack of a significant difference between the 3 hr. and 5 min. series is not surprising because, from the evidence of leaf damage, many individuals in the former spent only a few minutes actually feeding. It is doubtful if duration of source feeding had much effect on the proportion of infections produced in the initial sets of test plants, increased efficiency being more evident later on; though even after feeds of less than 5 min., transmissions were regularly obtained up to 7 days. The lower initial percentage obtained in the 3 hr. series compared with the under 5 min. one seems anomalous, but is probably attributable to the behaviour of the beetles. After 24 hr. starving, most fed voraciously when first placed on the source plants, and after a very short source-feeding period continued to do so when moved to test plants. Individuals allowed access to infected plants for 3 hr. were comparatively replete when transferred, and usually fed less. This explanation involves an assumption, which is justifiable (see below), that longer test feeding increases the chances of infection.

TABLE 3. *Transmitting efficiency for varying amounts of source and test plant tissue consumed*

		Test plant tissue eaten (sq.mm.)			%
		1-30	31-70	>70	
Source plant tissue eaten (sq.mm.)	1-30	*13/54	22/46	25/36	44
	31-70	7/25	14/35	8/13	40
	>70	8/23	9/15	15/24	52
	%	27	47	66	

* Numerators give plants infected, out of totals given by denominators.

Table 3 gives the results of trials designed to show how vector efficiency is affected by the duration of test feeding. This was assessed indirectly by measuring the area eaten from the simple leaves of young seedlings after confining the beetles on them for varying periods, a much quicker procedure than timing the intermittent feeding of individual vectors. Only one set of test plants was used this time, the insects being transferred to them directly after source feeding. It was also necessary to take into account variation in the preceding source feeds, and the amount of tissue eaten from the infected plants was measured. As the observations for some combinations of the two independent variables were few, the data were coarsely grouped in a 3×3 table. The transmissions in each 'cell' were calculated as percentages, which were subjected to angular transformation (Snedecor, 1946). An analysis of variance on the transformed percentages shows that the amount of source feeding had no significant effect, whereas larger amounts of test feeding significantly increased the number of infections ($P=1\%$), as would be expected in view of the prolonged retention of infectivity by the beetles. The variation due to test feeding was subdivided into its linear component and the component for curvature. The complete non-significance of the latter indicates that transmissions

for the three test-feeding intervals show a uniform rise. When the beetles damage the test plants excessively, destroying tissue they have previously infected, some reduction in the number of transmissions might be expected. There was, however, no indication of such an effect when they remained on the plants up to 24 hr., during which time most ate over 1 sq.cm. of leaf and some as much as 2.5 sq.cm.

Another serial experiment, involving prolonged source feeding and 24 hr. consecutive periods on the test plants, yielded results comparable with those in the right-hand column of Table 1, except that infections were not obtained beyond 12 days after source feeding. It is possible that the direct effect of longer test-feeding periods was counterbalanced by the resulting more rapid elimination of virus from the vector.

Trials were made to compare the efficiencies of unstarved vectors and those starved for 24 hr. before source feeding, conditions being otherwise identical. All were caged on the source plants for 1 hr., during which totals of forty-two starved and thirty-nine unstarved beetles each fed for a few minutes. These were transferred to test plants for 24 hr., the remainder being rejected. In three out of five trials the unstarved vectors produced a higher proportion of infections than the starved ones, and the total percentages of test plants infected were 77 and 71 respectively; even though the latter consumed on an average 5% more leaf tissue during the source-access period. It was not possible to make these experiments with beetles entirely comparable with those made by workers investigating the effect of preliminary starving on plant virus transmission by aphids (Bawden, 1950). A source-access period of 1 hr. permitted some unstarved beetles to fast for a time of their own volition; but a shorter period would have entailed a disproportionate number of rejections amongst them, because of failure to feed.

Dale (1949) showed that *Ceratoma ruficornis* readily transmits cowpea mosaic when fed for a few minutes successively on infected and healthy plants. Similarly, the results in the left-hand column of Table 1 suggest that, if there is any delay in the development of infectivity by the vector, this must be less than about 3 hr. It might be suggested, however, that rapid transmission occurs solely by mechanical contamination of the vectors' mouthparts, rather than in the manner operative for subsequent infections. However, the possibility of a latent period was ruled out by the results of a further experiment. Some previously starved beetles were divided into four groups: those in the 1st were placed for $\frac{1}{4}$ – $\frac{1}{2}$ hr. on infected leaves and the ones that fed were transferred directly to a succession of six sets of healthy plants, being allowed to remain for 1 hr. on each; the 2nd, 3rd and 4th groups were treated similarly, except that they were made to fast for 6, 12 and 18 hr. respectively between source feeding and transference to the first set of test plants. The routine had to be started at a different time for each group of vectors to enable all source and test feeds to be given in daylight, for these insects do not feed during darkness. Infections were obtained for every set of test feeds, covering a 24 hr. period after source feeding (Table 4).

TABLE 4. *Serial transmissions covering a 24 hr. period*

Fasting period between source and test feeds					Percentages of test plants infected ($\frac{1}{4}$ - $\frac{1}{2}$ hr. source-access periods)			
					Group I 0 hr.	Group II 6 hr.	Group III 12 hr.	Group IV 18 hr.
Successive hourly transfers to healthy test plants					1	11	31	20
					2	13	6	13
					3	16	6	27
					4	20	12	17
					5	15	6	19
					6	5	6	6
								11

MECHANISM OF TRANSMISSION BY BITING VECTORS

Until recently, it was generally assumed that when a biting insect behaves as a virus vector, it only does so by carrying on its mouthparts infective juice from diseased to healthy plants. This type of transmission is to be expected occasionally with any sap-transmissible virus, especially if highly infectious, and is probably operative with all the examples cited in the Introduction.

However, when a biting insect is an efficient vector and remains so for several days after feeding on diseased material, some additional transmission mechanism must be involved. Markham & Smith (1949) suggested that the vectors of turnip yellow mosaic transmit the virus by regurgitating infective juice from the foregut. They pointed out that all (with the possible exception of *Leptophyes punctatissima*) lack salivary glands and probably regurgitate to aid the ingestion of leaf tissue during feeding; this can happen (in *Phyllotreta* spp. and *Phaedon* larvae at least) because the vectors have no oesophageal valve. Markham & Smith inferred that caterpillars might fail to transmit the virus because they have salivary glands.

This feeding mechanism may be expected to lend itself to the dissemination of readily sap-transmissible viruses, provided they resist inactivation by enzymes of the foregut. There is little doubt that squash mosaic (Freitag, 1941*a, b*), and the cowpea mosaics of C. E. Smith (1924) and the writer (which may be closely related or even identical), are also transmitted in this way. Smith produced infection by inoculating plants with regurgitated juice or abdominal contents from *Ceratoma trifurcata*, but the significance of these results seems to have been overlooked. Cowpeas have been infected with mosaic in Trinidad by inoculation with macerated bodies of infective *C. ruficornis*, and very readily with regurgitated juice. Dr F. J. Simmonds (personal communication) finds that this beetle, like *Phyllotreta* and *Phaedon*, has neither functional salivary glands nor an oesophageal valve. Freitag (1941*b*) recovered the virus of squash mosaic from crushed individuals of *Diabrotica trivittata* and *D. soror* previously fed on diseased material, from *D. trivittata* after 5 days' feeding on healthy plants. The limited retention of infectivity by vectors of these viruses is consistent with gradual elimination of virus from the foregut. For

cowpea mosaic (at least) there is no evidence of inactivation by enzymes, as samples of infective juice stored at glasshouse shade temperatures (23–31° C.) lose their infectivity within three days—much sooner than vectors become non-infective. Regurgitation, rendered less effective by fairly rapid inactivation of virus in the foregut, may play a part in the transmission of tobacco mosaic by *Melanoplus differentialis*, though this virus is so highly infectious that contamination of the mouthparts may entirely account for the results obtained by Walters (1951).

Cowpea mosaic, squash mosaic and turnip yellow mosaic viruses are persistent (Watson & Roberts, 1939; Bawden, 1950), but contrast with some others in this heterogeneous group that are retained by their vectors, after source feeding, for life. The persistent viruses of aster yellows (Maramorosch, 1951), clover club leaf (Black, 1950) and probably that of rice stunt (Fukushi, 1940), multiply in their leaf-hopper vectors, the last two being passed on through the eggs to successive generations. Except for tomato spotted wilt virus, which becomes inactivated much more rapidly *in vitro*, the ones with biting vectors are the only persistent viruses that are readily sap-transmissible. Delay in the development of infectivity by their vectors has been reported for many persistent viruses, and a preliminary trial by Markham & Smith (1949) suggested that *Phaedon cochleariae* larvae may not be able to transmit turnip yellow mosaic until about 24 hr. after source feeding. There is no such delay with *Ceratoma ruficornis* and cowpea mosaic, and there seems little reason for it if transmission is accomplished by regurgitation from the foregut, for this lies close to the mouthparts. Further information on this point for turnip yellow mosaic and squash mosaic would be of interest. Cowpea mosaic virus, like all persistent ones, is transmitted no more readily by vectors starved before source feeding.

It is of interest that aphids, the commonest vectors of plant virus diseases, seem unable to transmit cowpea mosaic and turnip yellow mosaic (Markham & Smith, 1949); while of the viruses studied by Freitag (1941*a*), some seem to be almost exclusively transmitted by aphids and others (including squash mosaic) by beetles. Similarly, among viruses that are transmitted less efficiently by biting insects, tobacco ring-spot, potato virus *X* and the Trinidad egg-plant mosaic, cannot be transmitted by aphids so far as is known, and tobacco mosaic, in spite of its highly infectious nature, is not normally transmitted by them. Aphids have a suboesophageal valve; so when they do behave as vectors this cannot depend on regurgitation from the gut. The most likely explanation of their failure to transmit infectious viruses such as turnip yellow mosaic, cowpea mosaic, egg-plant mosaic, tobacco ring-spot and potato *X*, is that these are prone to inactivation by some substance which aphids produce. Occasional aphid transmissions like those reported for squash mosaic (Freitag, 1941*a*) and tobacco mosaic (Hoggan, 1931) might then result from small quantities of virus escaping inactivation. The secretion of an inactivator by feeding aphids was postulated by Watson (1938) to explain their greater efficiency as vectors of certain non-persistent viruses after being permitted

only short source feeds, following enforced fasting. Perhaps the viruses under consideration exhibit the ultimate degree of non-persistence with aphids; but persist in certain biting insects because either the latter produce no inactivator or, if they do, the viruses do not come into contact with it. Additional information on the transmission of squash mosaic by aphids would be of interest in this connection.

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QUANTITATIVE STUDIES ON THE TRANSMISSION OF CABBAGE BLACK RING SPOT VIRUS BY *MYZUS PERSICAE* (SULZ.)

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Factors affecting the transmission of cabbage black ring spot virus by *Myzus persicae* (Sulz.) were studied quantitatively using the local lesions produced on tobacco leaves. Aphids prevented from feeding for 15 min. or more, before feeding for a few minutes on an infected plant, caused more infections than unfasted aphids. Fasted aphids acquired virus from infected plants in feeding times as short as 10 sec., and infected healthy plants in test-feeding times of 5 sec. Increasing test-feeding times to 30 min. increased the numbers of infections. Increasing infection-feeding times from 10 sec. to 5 min. had little effect, but increasing to more than 5 min. greatly reduced the number of transmissions. This reduction was partly offset if the aphids were prevented from feeding continuously while on the infected plants. With undisturbed infection-feeding periods of 15 min. or longer, previously fasted aphids caused no more infections than unfasted aphids.

Infective aphids lost their ability to produce lesions more rapidly when feeding than when fasting.

Winged and wingless aphids were equally efficient vectors.

A virus found in swede plants on Rothamsted farm is thought to be related to that listed in the *Review of Applied Mycology* (1946) under the name cabbage black ring spot virus (CBRSV). The experiments described in this paper were made to study its transmission by the aphid *Myzus persicae* (Sulz.).

MATERIALS AND METHODS

Stock cultures of the virus were maintained in turnip plants (*Brassica rapa* L. var. Early Snowball), and systemically infected turnip leaves were used as the source of infection. Tobacco plants (*Nicotiana tabacum* L. var. White Burley) were used as test plants, and the aphids were placed on the two second non-hairy leaves where they feed readily. Infection of tobacco leaves by inoculation with sap or by infective aphids produces small necrotic lesions within 5-7 days (Kvíčala, 1949). These are readily counted and provide a more accurate method for quantitative studies than systemic infections, which may result from one or many separate infections.

The aphids were reared and handled according to the methods described by Watson (1936, 1938). The special terms referring to the techniques used in the aphid-transmission experiments are as follows:

Preliminary fasting time: period without food before infection feeding.

Infection-feeding time: period during which an aphid feeds on an infected plant.

Test-feeding time: period during which an aphid feeds on a healthy plant after infection-feeding.

Post-infection fasting time: period without food after infection feeding.

Consecutive feeding: test-feedings of equal duration on a succession of healthy plants after infection feeding.

SYMPTOMS AND HOST RANGE

Symptoms appear in turnip plants 10–15 days after inoculation with CBRSV. The younger leaves become crinkled and later irregular raised patches of dark green tissue are formed, giving a blistered appearance which persists during the life of the plant. The blistering is sometimes preceded by coarse vein clearing and interveinal mottling; it is generally confined to the outer leaves. Young plants are sometimes killed, and older plants are much stunted.

The virus was transmitted mechanically and produced systemic symptoms in the following plants: *Brassica chinensis* L., *B. caulorapa* Pasq., *B. hirta* Moench., *B. muralis* Boiss., *B. oleracea* L. var. *acephala* DC., *B. oleracea* L. var. *botrytis* L. Extra Early Roscoff, *B. oleracea* L. var. *botrytis* L. St. George, *B. oleracea* L. var. *botrytis* L. Cambridge No. 11, *B. oleracea* L. var. *bullata* L., *B. oleracea* L. var. *capitata* L. January King, *B. oleracea* L. var. *gemmifera* Zenker. Cambridge No. 1, *B. oleracea* L. var. *italica* Plenck, *B. pekinensis* Rupr., *Capsella Bursa-pastoris* Medic., *Cichorium endivia* L., *Matthiola incana* R. Br. var. *annua*. Voss., *Papaver nudicaule* L., *P. Rhoeas* L., *P. somniferum* L., *Petunia hybrida* Vilm., *Verbena hybrida* Voss. and *Zinnia elegans* Jacq.

Local necrotic lesions occurred in *Nicotiana rustica* L., *N. glutinosa* L., *N. tabacum* L. and *Lycium halimifolium* L.

PHYSICAL PROPERTIES

Some of the properties of CBRSV were determined by inoculating *Nicotiana tabacum* or *N. glutinosa* with infective turnip sap.

The dilution end-point was 1/1000. The thermal inactivation point (10 min. heating) was around 60° C., but much infectivity was lost at temperatures below the thermal inactivation point. Infectivity was retained at room temperature (about 17° C.) for 48 hr. but not for 72 hr. Similar properties have been reported previously for viruses attacking cruciferous plants, and it is probable that CBRSV resembles turnip virus 1 (Hoggan & Johnson, 1935) and turnip mosaic virus (Tompkins, 1938).

THE TRANSMISSION OF CABBAGE BLACK RING SPOT VIRUS

BY *MYZUS PERSICAE*

The virus used by Kvičala (1948, 1949) under the name cabbage mosaic is also probably CBRSV. Kvičala found that in its transmission by aphids this virus behaved like the non-persistent viruses studied by Watson (1936, 1938) and Watson

& Roberts (1939). In the following experiments the effect of varying fasting and feeding times on the transmission of CBRSV were further studied.

Effect of varying preliminary fasting times

In two experiments on the duration of preliminary fasting times, groups of *M. persicae* were fasted for differing periods before a short infection-feeding time of 2 min. Thirty aphids were tested for each treatment on five occasions. Groups of five aphids were placed on two leaves of each tobacco plant using three plants per treatment. The test-feeding time was 24 hr.

For Exp. I the preliminary fasting times were 0 min., 15 min., 1 hr., 4 hr. and 16 hr. The transformation log ($n+c$) was applied to the data— n being the number of local lesions. The best value for the constant c was found to be 10 according to the method described by Kleczkowski (1949). The number of lesions increased significantly between 0 and 15 min. and between 15 min. and 1 hr. but later increases were small and not statistically significant. (Table 1).

TABLE 1. *Effect of varying periods of preliminary fasting on the transmission of cabbage black ring spot virus by Myzus persicae. Five aphids per leaf*

Experiment I						
Preliminary fasting times						
	0 min.	15 min.	1 hr.	4 hr.	16 hr.	Mean
Actual nos. of lesions	37	81	134	158	161	114.2
Means log ($n+10$)	1.23	1.41	1.56	1.61	1.62	1.49
S.E. of treatment mean (± 0.041)						
Experiment II						
Preliminary fasting times (min.)						
	0	5	15	30	60	Mean
Actual nos of lesions	18	46	108	145	144	92.2
Means log ($n+10$)	1.13	1.28	1.49	1.58	1.59	1.41
S.E. of treatment mean (± 0.039).						

Exp. II (Table 1) confirmed that the main effect of starving occurs during the first 15 min. and that fasting for more than 30 min. has little further effect on transmission.

Effect of preliminary fasting and varying infection-feeding times

With the non-persistent viruses (Watson, 1938) the response to preliminary fasting disappears if the infection-feeding time is increased. Table 2 shows that this is also true with CBRSV. Aphids which had fasted for 4 hr. caused five to six times as many lesions as those unfasted when the infection-feeding time was five

minutes or less, but extension of the infection-feeding time to 15 min. or more greatly reduced the numbers of lesions in that fasted aphids caused no more than unfasted ones.

TABLE 2. *Effect of preliminary fasting and varying infection-feeding times on the transmission of cabbage black ring spot virus by Myzus persicae. Three aphids per leaf*

	Infection-feeding times (min.)											
	No preliminary fasting						4 hr. preliminary fasting					
	2	5	15	30	60	Mean	2	5	15	30	60	Mean
Actual nos. of lesions	15	17	15	4	1	10.4	91	86	19	6	5	41.4
Means log ($n+10$)	1.11	1.11	1.11	1.03	1.01	1.07	1.45	1.41	1.13	1.04	1.04	1.21
S.E. of treatment mean (± 0.025).												

Effect of disturbing aphids during or before infection-feeding times

Fasted and unfasted aphids were given infection feeds of 2 min. and 1 hr. All aphids were fed on detached infected turnip leaves in moist containers. Two groups received the 1 hr. infection feed. Of these, one group remained undisturbed, whereas each aphid in the other was disturbed every 5 min. and moved to a different part of the leaf. Twenty-five aphids were tested for each treatment on five occasions.

Preliminary fasting increased the numbers of lesions caused by the aphids which received a 2 min. infection-feeding time, but had no effect on either disturbed or undisturbed aphids which had 1 hr. infection feeding (Table 3). Disturbing the

TABLE 3. *Effect of disturbing Myzus persicae during the infection-feeding time. Five aphids per leaf*

	Infection-feeding times			
	Actual nos. of lesions caused by <i>Myzus persicae</i>			Mean
	2 min.	1 hr.	1 hr. disturbed every 5 min.	
No preliminary fasting	16	15	65	32
4 hr. preliminary fasting	111	11	49	57
Means log ($n+10$)				
No preliminary fasting	1.11	1.10	1.35	1.19
4 hr. preliminary fasting	1.49	1.08	1.29	1.29
S.E. of treatment mean (± 0.033).				

aphids during the 1 hr. infection feed greatly increased the lesion numbers, for both fasted and unfasted aphids. The disturbed aphids gave a total of 114 lesions, and the undisturbed 26.

To test whether this effect was due to the accumulation of short fasting times, part of the experiment was repeated, but a treatment was included in which aphids

were disturbed every 5 min. on a healthy turnip leaf for 1 hr. before a 2 min. infection feed. The treatments were therefore, 4 hr. preliminary fasting followed by: 2 min. infection feeding; 1 hr. infection feeding; 1 hr. disturbed every 5 min. on a healthy leaf before 2 min. infection feeding; 1 hr. disturbed every 5 min. on an infected leaf before final 2 min. infection feeding. One aphid was tested for each treatment on six tobacco leaves on five separate occasions.

TABLE 4. *Effect of disturbed periods of feeding on healthy and infected leaves before infection feeding. One aphid per leaf*

Treatment after 4 hr. preliminary fasting			
Infection-feeding times			
2 min.			1 hr.
1 hr. on infected leaf disturbed every 5 min		1 hr. on healthy leaf disturbed every 5 min.	None
None			
31	19	11	3

The data were few as only single aphids were used, but the results suggest strongly that whether the interrupted feeding took place on infected leaves, or whether on healthy leaves before 2 min. infection feed, numbers of lesions showed a great increase over those caused after 1 hr. continuous infection feeding (Table 4).

Effect of varying infection- and test-feeding times after preliminary fasting

To determine the minimum times required by *M. persicae* for infection and test feeding, the aphids were fasted for 4 hr. and then given infection feeds of 5, 10, 20 and 40 sec., and test feeds of 5, 10, 20 and 40 sec., in a factorial arrangement. In the first three treatments most aphids made a single puncture, but one or more were made by those in the 40 sec. group. Eight aphids were tested for each treatment on five separate occasions.

Table 5 shows that prolonging infection feeding beyond 10 sec. did not increase the number of infections, but prolonging test-feeding times beyond the minimum necessary, did.

TABLE 5. *Effect of varying infection- and test-feeding times after preliminary fasting on the transmission of cabbage black ring spot virus. One aphid per half leaf*

Actual nos. of lesions caused by <i>Myzus persicae</i>					
Test-feeding times (sec.)	Infection-feeding times (sec.)				Total
	5	10	20	40	
5	0	0	0	1	1
10	0	0	1	0	1
20	0	3	3	3	9
40	0	5	4	2	11
Total	0	8	8	6	22

Groups of *M. persicae*, which had fasted for 4 hr. and received an infection feed of 2 min., were given post-infection fasting times of 0, 5, 15, 30 and 60 min. before being placed on the test plants. Six groups of five aphids were used for each treatment on seven occasions. The lesion numbers fell by one-quarter within 30–60 min., which suggests that the second value in the previous experiment was rather high, and that infectivity falls steadily (Table 7).

Effect of 5 min. consecutive feedings by single aphids

Watson & Roberts (1940) showed that aphids could infect more than one host plant with *Hyoscyamus* virus 3, potato virus *Y* and severe etch viruses respectively. To test the effect of consecutive feedings with CBRSV individual aphids were moved to different tobacco leaves every 5 min. during a 1 hr. period. These aphids had a preliminary fasting time of 4 hr. and an infection feed of 2 min. Five single aphids were tested on five occasions. Thirteen aphids caused lesions during the first 5 min. and of these ten caused lesions during subsequent transfers (Table 8). Much of the infectivity was exhausted, however, after 35 min.

TABLE 8. *Local lesions obtained after 5 min. consecutive test feedings by single aphids*

Aphid no.	Consecutive feedings												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
1	1	0	0	0	0	0	0	0	0	0	0	0	1
2	1	0	0	0	0	0	0	0	0	0	0	0	1
3	1	0	0	0	0	0	0	0	0	0	0	0	1
4	1	1	0	0	0	0	0	0	0	0	0	0	2
5	1	1	0	0	0	0	0	0	0	0	0	0	2
6	0	1	0	0	0	0	0	0	0	0	0	0	1
7	2	0	1	1	0	0	0	0	0	0	0	0	4
8	2	0	0	1	0	0	0	0	0	0	0	0	3
9	0	1	0	1	0	0	0	0	0	0	0	0	2
10	2	1	1	1	1	0	0	0	0	0	0	0	6
11	1	2	1	1	1	0	0	0	0	0	0	0	6
12	1	0	0	0	0	1	0	0	0	0	0	0	2
13	0	0	0	1	0	1	0	0	0	0	0	0	2
14	1	0	0	1	0	1	1	0	0	0	0	0	4
15	1	0	0	0	0	0	1	0	0	0	0	0	2
16	1	0	0	0	0	0	1	1	0	0	0	0	3
17	0	1	0	0	0	0	1	0	0	2	0	0	4
18	0	0	0	0	0	0	0	0	0	0	1	0	1
Total	16	8	3	7	2	3	4	1	0	2	1	0	47

Seven aphids did not cause lesions. These are not included in the table.

Effect of varying numbers of infective Myzus persicae on the number of lesions

Groups of 1, 2, 5 and 10 fasting aphids were given an infection-feeding time of 2 min. and were tested on the two most suitable leaves of twelve healthy tobacco plants. Aphids were placed singly on every test leaf in turn and three plants were removed at random to form the group receiving one aphid per leaf. The process

was repeated till there were the required number of aphids on each series of plants. The total number of lesions (Table 9) increased with aphid numbers but the number of lesions per aphid decreased slightly with the larger aphid groups, so that the increases for five and ten aphids were slightly less than multiples of five and ten.

A possible explanation was that the first few aphids received their infection feed while the turnip leaf was newly detached, and aphids feeding later when the infected leaf was not in such good condition, possibly had less chance of becoming infective. To obviate this, aphids which had received their infection feed were placed on the test leaves alternately in successive groups of 1, 2, 5, 10 and 10, 5, 2 and 1. In this way the performance of the larger aphid groups in relation to the smaller groups was improved, though there was still a slight reduction in number of lesions per aphid in the 10-aphid group (Table 9, Trial 2).

A trial was then made on tobacco plants from which all leaves but one were removed, to prevent possible loss of lesions due to aphids feeding on other parts of the plant (Table 9, Trial 3). This, however, had no effect on the distribution of lesions between the different groups. The most likely explanation is that some lesions may have been the result of more than one infection-feeding puncture.

TABLE 9. *Effect of varying numbers of Myzus persicae on the numbers of lesions*

	No. of <i>Myzus persicae</i> per leaf				No. of infective aphids obtained in first treatment (out of 30)
	1	2	5	10	
Trial 1	41	82	176	302	18
Average no. of lesions per aphid	1.37	1.37	1.17	1.01	
Trial 2	31	66	171	282	18
Average no. of lesions per aphid	1.03	1.10	1.14	0.94	
Trial 3	32	52	151	278	19
Average no. of lesions per aphid	1.07	0.87	1.01	0.93	

Effect of varying numbers of infective Myzus persicae on the transmission of cabbage black ring spot virus to whole plants

Similar groups of *M. persicae* were placed on seedling turnips to determine the effect of varying numbers of aphids on the transmission of CBRV to whole plants, using the method described for Trial 2.

The results showed that the number of infected plants was increased by the larger numbers of infective aphids. This increase was not proportional to the increase in aphid numbers but agrees reasonably well with values based on the expectation that a single infective puncture causes infection. The reduction of percentage of infection obtained with higher numbers is almost completely accounted for by loss of potential infectivity caused by more than one aphid in a group giving an infective puncture, and suggests that variation in susceptibility between turnip plants is small.

*Comparison of the efficiency of alate and apterous adults and nymphs
of Myzus persicae as vectors*

Adults and nymphs of both forms of *M. persicae* were fasted for 4 hr. and given an infection feed of 2 min. before being placed on the test plant. The adults of the alatae were active, tending to leave the test plant and fly to the top of the lamp glass used to enclose the plant. The apterae and young alatae remained on the leaves of the test plants, feeding continually during the test-feeding time of 1 hr.

The results showed that alatae produced fewer lesions than apterae but the difference was not statistically significant. This might have been attributable to migrating tendencies in the alatae, preventing them from remaining on the leaf. However, the alate nymphs also caused fewer lesions than the apterous nymphs which suggests that there may be some difference between them, although it is rather small.

DISCUSSION

The ease with which *M. persicae* could transmit CBRSV greatly increased when the aphids had been given periods of preliminary fasting before feeding on the source of infection. Sylvester (1949), Chaudhuri (1950) and Bradley (1952), working with beet mosaic virus, pea mosaic virus and henbane mosaic virus respectively, suggest that the influence of fasting on vector efficiency is fully developed within 15 min. Watson (1938), Watson & Roberts (1939) and Kassanis (1941) have shown that with some non-persistent viruses the increase may continue for several hours but the rate of increase decreases rapidly with time. The results with CBRSV also suggest that although the largest effect of fasting is apparent soon after the removal of the aphids from the food plants, the increase probably continues for some time, though at a rapidly decreasing rate.

After preliminary fasting, increases in the infection-feeding times decreased the numbers of lesions caused on tobacco leaves by the groups of *M. persicae* transmitting CBRSV. This behaviour of *M. persicae* is similar to that reported by Watson (1938, 1946) and Kassanis (1941) with other non-persistent viruses. Usually the effect of preliminary fasting is negligible after 1 hr. infection feeding, but when the aphids are disturbed frequently during a 1 hr. infection feed, their infectivity is less reduced than when feeding continuously. This effect was the same for aphids which had not fasted previously. During these periods, the aphids may have been feeding repeatedly in epidermal tissues, where the virus is probably more readily available to them, instead of penetrating to the phloem where virus may be less concentrated (Bradley, 1952). A similar, though smaller, effect was also produced when the period of disturbed feeding took place on a healthy leaf before the infection feed. This would suggest that either there was some cumulative effect of the short fasting periods between the disturbed feeds, or it was only the final penetration which caused aphids to become infective. There seemed to be some influence, however, of the previous penetrations on infected leaves because aphids thus

disturbed gave more lesions than those which were disturbed on healthy leaves during the pre-infection feeding period. This point requires some further investigation.

During successive short test feedings individuals of *M. persicae* were able to cause lesions on a series of healthy leaves after a single infection feed. With other non-persistent viruses (Kassanis, 1941; Watson, 1946; Watson & Roberts, 1940), most aphids infected the first plant on which they fed, and the number of plants to become infected decreased with successive feedings. Similarly, in transmitting CBRSV, *M. persicae* causes more lesions on the first leaves and little infectivity is retained after 35 min. CBRSV differs from most other non-persistent viruses which have been tested, in the wider intervals of time between successful punctures. These aphids frequently caused no infections for four or five transfers, and there were sufficient long delays before any infection was caused at all to account for the increases in vector efficiency which occurred with increasing test-feeding time as determined previously (Table 6).

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THE SYSTEMIC INSECTICIDAL ACTION OF SODIUM FLUOROACETATE AND OF THREE PHOSPHORUS COMPOUNDS ON THE EGGS AND LARVAE OF *PIERIS BRASSICAE* L.

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(With 1 Text-figure)

Four compounds, *bis*dimethylaminophosphonous anhydride (*anhydride*), *bis*(dimethylamino)fluorophosphine oxide (*oxide*), diethyl paranitrophenyl phosphate (E600) and sodium fluoroacetate (*acetate*), previously shown to have systemic insecticidal activity against aphids, have now been tested against the eggs and larvae of *Pieris brassicae* L.

The anhydride proved to have very little toxic action on *Pieris*, but the other three compounds showed both contact toxicity and systemic insecticidal action when taken up by the roots of cabbage plants from solution and from soil. The acetate, but more especially the E600, also showed systemic action following application to the leaves.

In all cases the order of decreasing toxicity was E600 > acetate > oxide > anhydride. E600 is the only compound which is outstandingly toxic to *Pieris* eggs and larvae. It has the added interest that when watered on to the roots of cabbage plants it kills larvae in egg batches on the leaves. Death takes place at the stage when the larvae are biting through the shells. The oxide and acetate proved to be surprisingly innocuous.

Unless it is felt to be too poisonous E600 is worthy of consideration as an insecticide against *Pieris* eggs and larvae, since it is highly effective as a contact insecticide and also has some systemic action.

Most of the published work on systemic insecticides concerns their effectiveness against aphids or mealybugs. There is apparently no reason, however, why they should not be used to control biting and chewing insects and this possibility has been investigated using the larvae of *Pieris brassicae* L.

The materials used in this investigation were (i) *bis*dimethylaminophosphonous anhydride called the *anhydride*, (ii) *bis*(dimethylamino)fluorophosphine oxide, called the *oxide*, (iii) diethyl *p*-nitrophenyl phosphate, called E600, and (iv) sodium fluoroacetate, called the *acetate*. The properties and origins of these materials have already been described (David & Gardiner, 1951).

METHODS AND EQUIPMENT

The insects and plants were kept and the experiments were conducted in the greenhouse previously described (David & Gardiner, 1951).

Pieris brassicae L. was chosen as the test insect because it proved to be easy to

rear all the year round in very large numbers (David & Gardiner, 1952). All the insecticidal experiments were carried out with young cabbage or Brussels sprout plants, 4–8 in. high.

The *Pieris* larvae were confined on the treated plants in wire gauze cages. The cages were cylindrical, 4 in. in diameter and 6 or 7 in. high. The only opening was at the bottom, where the end of the wire gauze was soldered to a $\frac{1}{2}$ in. brass ring cut from tubing. During the test the cages rested on a board in which slots had been cut at 5 in. intervals. Each slot was 1 in. long and wide enough to take the stem or the petioles of several leaves of the test plant. To prevent insects escaping the stems or petioles were packed in the slots with cotton-wool or sponge rubber.

The percentage number of larvae killed was determined. Owing to the rather small quantities of some of the systemics available relatively few cabbage plants could be treated, and, as larvae consume large quantities of cabbage, the number of larvae had also to be restricted. The percentage kill figures were therefore based on about 20–40 larvae in groups of 5 or 10.

Phytotoxicity was assessed on the plants as follows:

Normal	N	Not different from controls.
Trace	T	Slight wilting or marginal leaf scorch or scattered spotting.
Moderate	M	Extensive wilting and/or large areas of the leaves scorched and some entirely destroyed.
High	H	Plants almost entirely killed though the undeveloped leaves at the growing point may be green.
Dead	D	Plant dead.

RESULTS

Tests with ova and newly hatched larvae

(1) *Contact action*

As systemic insecticides are usually applied by spraying on to the leaves of plants an appreciable part of their action may be brought about by direct contact. The importance of this factor has been investigated by dipping tests.

The leaves of young cabbage plants bearing batches of ova were dipped into solutions of the insecticides containing 0.1 % 'Teepol' as a wetting agent and kept at 18–20° C. After dipping the plants were laid on their sides until dry to prevent the solutions running down to the roots. When treated, eggs were all within 48 hr. of hatching. Detailed examination of eggs showed that the larvae developed fully, but that they were killed in the stage when they had just pierced the shell. Figures for the approximate percentage of eggs from which larvae fail to emerge and for the kill of the young larvae in a series of experiments are presented in Table 1. It can be seen that only E600 prevents hatching. At the highest concentration tested the acetate proved to be toxic to the newly hatched larvae which died soon after they had begun to feed on the treated leaves.

TABLE 1. *Cabbage leaves bearing eggs dipped in the insecticide solutions*

Dipped 12 Dec. 1951. Hatch complete 14 Dec. 1951

Material	Concentration (% v/v)	Percentage of eggs not hatching	Percentage kill of larvae on 5th day after hatching
Anhydride	0.2	0	0
	0.1	0	0
Oxide	0.2	0	0
	0.1	0	0
E 600	0.01	100	—
	0.005	100	—
	0.001	50	100
	0.0001	5	0
Acetate	0.1 (w/v)	0	100
	0.05	0	10
	0.01	0	0
Control (0.1 % 'Teepol')	—	0	0

(2) *Systemic action following absorption by the roots*

(a) *Plants in solutions.* Young cabbage plants on which batches of eggs had been deposited were removed from the soil and their roots were carefully washed. The roots of these plants were then transferred to insecticidal solutions. It is impossible to avoid damaging the roots during this process, and to prevent serious wilting it is necessary to set up the experiment in a shady position. The results obtained are shown in Table 2. It can be seen that the eggs hatched on the plants treated

TABLE 2. *Cabbage plants bearing eggs 2 days old with their roots placed in solutions of the insecticides. Hatching commenced 4 days later*

Date: 7 Dec. 1951

Material	Concentration (% v/v)	Percentage of eggs not hatching	Percentage kill of larvae on 3rd day after hatching	Condition of plants after 5 days in solutions
Anhydride	0.2	5	0	T
Oxide	0.1	0	100	T
	0.01	0	0	T
E 600	0.05	100	—	D
	0.01	100	—	H
	0.001	80	100	M
Acetate	0.1 (w/v)	10	100	T
	0.01	5	25	T
Control	—	5	0	T

systemically with the anhydride, oxide and acetate. The larvae fed normally on the anhydride-treated plants but were killed on plants fed with 0.1 % oxide and acetate solutions.

The most notable result of these experiments, however, was the observation that no larvae emerged from *Pieris* eggs on the leaves of cabbage plants which had absorbed E600 systemically from the root. The effect cannot be described as ovicidal action since the larvae always developed fully within the egg and were only killed at the stage when they pierced the egg shells. The precise mode of action concerned is being investigated, and it is hoped to discuss the subject in a later paper.

In these experiments the test plants showed rather a large degree of leaf damage which was, at least partly, caused by transferring them from soil to culture solution. Plants treated in soil remained normal (Table 3).

TABLE 3. *Insecticide solutions watered on soil in which cabbage plants, bearing 24 hr. old ova, are growing. Eggs hatched when 7 days old*

Material	Quantity present in 20 c.c. solution	Percentage of eggs not hatching	Percentage kill of larvae 3 days after hatching	Conditions of plants 5 days after treatment
Anhydride	0.04 (c.c.)	0	0	N
Oxide	0.02 (c.c.)	0	0	N
E600	0.01 (c.c.)	100	—	N
	0.001	95	100	N
Acetate	0.02 (g.)	0	100	N
	0.002	0	0	
Control	—	0	0	N

(b) *Plants in soil.* The young cabbage plants bearing batches of *Pieris* eggs were treated by applying 20 c.c. of the insecticidal solutions to the compost in which they were growing. Again only E600 prevented the eggs from hatching, and only the E600 and the acetate-treated plants were toxic to the young larvae (Table 3). There was no evidence of phytotoxicity.

(3) *Systemic action following absorption by the leaves*

(a) *Penetration through the leaves.* Experiments were set up to determine whether the insecticides applied to the upper surfaces of cabbage leaves could affect eggs deposited on the undersurfaces (or vice versa) or young larvae before they ate through the leaves. Because of the low toxicity of the oxide and anhydride to *Pieris* eggs and larvae just reported only the acetate and E600 have been tested. Unless a toxic effect is shown by this technique a systemic action involving translocation greater distances within the plant cannot be expected. As the applications of insecticide are made close to the egg batches it is necessary to demonstrate that a fumigant action is not occurring. Batches of *Pieris* eggs laid on filter-papers or on cabbage leaves were accordingly enclosed either with leaves of other plants treated systemically or by repeated brushing with 0.01 % E600 or with capsules of the same solution. All the eggs hatched normally. The acetate was not tested in this way.

In the experiments on penetration the adults were allowed to lay on one or two leaves of each cabbage plant while the rest of the plant was masked. Twenty-four hours later the insecticidal solutions, containing 0.1 % Teepol, were brushed over about 1 sq.in. of the leaf surface on the opposite side to that on which the eggs were laid and directly over or under the egg batch. The applications were made four times a day for 2 days. In some cases there were several batches of eggs on the leaves and not all of these were treated. The results obtained with E600 are set out in Table 4. The acetate was tested at the same time, but as it had no effect on eggs at 0.1 % the results are not reported.

TABLE 4. *Systemic action of E600 brushed on one surface of a cabbage leaf on eggs of Pieris on the other surface*

Date: 11 Dec. 1951

Concentration (% v/v)	Plant no.	Leaf no.	Surface on which eggs laid	No. of egg batches		Percentage of eggs not hatching
				Treated	Untreated*	
0.01	1	1	Under	2	—	100
			Under	—	1	95
		2	Under	1	—	100
		1	Under	1	—	100
		3	Upper	2	—	100
		4	Upper	1	—	100
0.001	5	1	Upper	—	1	100
			Under	3	—	100
		2	Under	—	2	100
			Under	2	—	100
		6	Under	—	3	95
			Upper	1	—	100
0.0001	7 and 8	1	Upper	1	—	100
			Upper	1	—	100
		2	Upper	—	1	0
			Upper	—	1	0
Control	9 and 10	1	Under	1	—	0
		2	Under	—	—	0

* *Treated* means that insecticide was applied to the reverse side of the leaf to that on which the eggs were laid exactly opposite to the point of attachment of the eggs. Conversely, *Untreated* means that no insecticide was applied.

It can be seen that when solutions of E600 were applied to cabbage leaves batches of *Pieris* eggs on the reverse side did not hatch even if the application was not made to that part of the reverse side which was opposite to the point of attachment of the eggs. Movement of E600 from one part of the leaf to another was most clearly demonstrated when the treated region was nearer the petiole than the batch of eggs affected, that is E600 seemed to move more readily outwards from the base towards the tip of the leaf.

(b) *Translocation from older to younger leaves.* The five lower leaves of young cabbage plants were treated in January 1952, being brushed four times daily for 4 days with 0.01 % E600 containing 0.1 % Teepol as a wetter. On the fifth day

these leaves were cut off and adult *Pieris* were allowed to oviposit on the untreated younger leaves. Six days later all the eggs hatched, so that under these conditions toxic quantities of E 600 did not reach the eggs.

In the experiment just described there was an interval of 6 days between the last treatment of the older leaves and the hatching of the eggs on the untreated leaves. As it seemed possible that this period was too long for the E 600 to exert its maximum effect a different procedure was tried. In this four or five outer leaves of a young

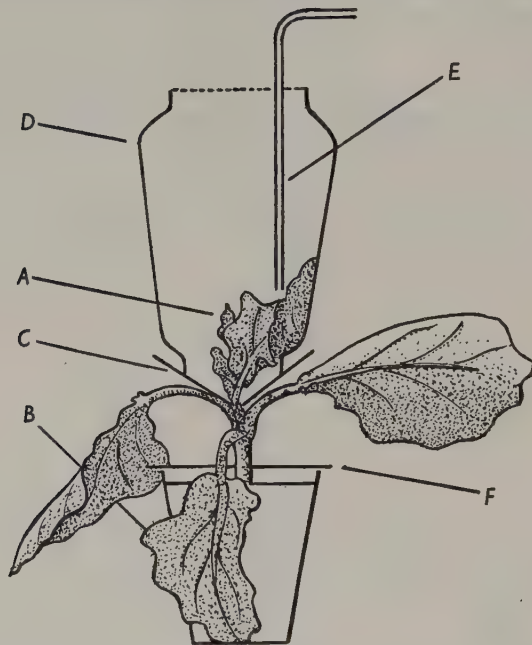


Fig. 1. Method employed for demonstrating the translocation of insecticide from treated to untreated leaves. The untreated leaves, *A*, are screened from the leaves brushed with insecticide, *B*, by the lamp glass, *D*, resting on the filter-paper cone, *C*. The lamp glass is supplied with compressed air through 'E'. Filter-paper disks, *F*, prevent the insecticide applied to the leaves dripping on to the soil in which the plant is growing.

cabbage plant were brushed with E 600 during the last 4 days of the hatching period of the eggs laid on the two inner leaves. The detailed procedure was as follows: the leaves of the young cabbage plant were screened with muslin, leaving only the two youngest exposed and on these eggs were collected. When the eggs were 2 days old the leaves bearing them were caged in lamp glass separately from the rest of the plant (Fig. 1), and the inside of each glass cage was supplied with a slow stream of compressed air. In this way it was possible to prevent the E 600 brushed on to the older leaves reaching the eggs except via the plant. Although the results were variable definite evidence was obtained by this procedure that after repeated applica-

tions E600 was translocated from treated to untreated leaves in sufficient quantity either to prevent *Pieris* larvae emerging from the eggs or to kill a high percentage of the newly emerged larvae (Table 5).

TABLE 5. *Systemic action of E600 in 0.01 % v/v solution with 0.1 % v/v Teepol brushed onto the outer leaves of young cabbage plants on Pieris eggs and young larvae on the inner leaves. In all cases one immature leaf and the unexpanded crown leaves were left untreated*

Plant no.	Treatments			Effect on eggs and larvae	
	No. of days given	Total no.	No. of leaves treated	Percentage of eggs not hatching	Percentage kill of emerging larvae after one day on leaf
1	8	25	4	0	100
2	8	25	5	10	98
3	5	16	4	25	66
4	5	16	5	100	—
5	Control			0	0

In a further series of tests one application of 0.1 % v/v E600 containing 0.1 % Teepol was given to the older leaves of cabbage plants in place of repeated applications of more dilute solutions. Although the insecticide was applied at intervals of from 1 to 4 days before the eggs on the young leaves were due to hatch, all the larvae emerged normally and none of them were definitely affected by feeding on the untreated leaves for the following 3 days. It is clear that insufficient E600 is translocated under these conditions to prevent the eggs on the untreated leaves from hatching, but, as will be shown later, one suitably timed application can have an effect on actively feeding larvae.

TESTS WITH SECOND- AND THIRD-INSTAR LARVAE

(1) *Contact action*

Under practical conditions larvae, like ova, are exposed to the contact action of systemic insecticides, and simple dipping tests were therefore carried out in order to obtain an approximate idea of the contact toxicity of the four compounds. The larvae were covered with the solutions for half a minute, which were then drained off and the larvae were caged with young cabbage plants. Teepol at 0.1 % was used as a wetter in all solutions and alone as a control. The results obtained are shown in Table 6. Where no kill was observed only the highest concentration tested is shown. It can be seen that E600 is by far the most effective contact insecticide.

(2) *Feeding on dipped foliage*

In these tests five larvae were allowed to feed on the leaves of young cabbage plants which had been dipped in solutions of the insecticides again containing 0.1 % Teepol as a wetting agent. After dipping, the plants were placed on their

sides, and allowed to dry, so that none of the solutions reached the roots. The results obtained are summarized in Table 7. In one experiment the larvae on the anhydride- and oxide-treated plants were supplied daily with a freshly dipped plant. In this way they had an ample supply of treated foliage available, but they showed no evidence of poisoning at the end of 5 days. Once again E600 was outstandingly more toxic than the other compounds, though the acetate killed some larvae at much higher concentrations. Both compounds greatly reduced the quantity of foliage eaten by the larvae.

TABLE 6. *Third-instar Pieris larvae dipped in solutions of the insecticides at 20° C.*

Date: 20 and 25 July 1951

Material	Concentration (% v/v)	Percentage of larvae dead on day	
		2	5
Anhydride	0.2	0	0
Oxide or Acetate (w/v)	0.1	0	0
E600	0.1	100	—
	0.01	100	—
	0.001	0	0
Control	—	10	10

The mortality figures are corrected for deaths among the controls.

TABLE 7. *Third-instar Pieris larvae placed on dipped cabbage leaves*

Date: 17 Dec. 1951

Material	Concentration (% v/v)	Percentage of treated foliage eaten on 2nd day	Percentage of larvae dead on day	
			2	5
Anhydride	0.2	90	0	0
	0.1	100	0	0
Oxide	0.1	100	0	0
	0.05	90	0	0
E600	0.01	< 10	100	—
	0.001	< 10	50	90
	0.0001	50	0	0
Acetate	0.2 (w/v)	< 10	70	100
	0.1	< 10	0	20
Control	—	90	0	0

(3) Systemic action following absorption by the roots

(a) *Plants in solutions.* The young cabbage plants were unpotted in the usual way and their roots were placed in solutions of the insecticides which were renewed after five days. On the first or fourth day after treatment had begun five *Pieris* larvae were enclosed with each plant in the wire gauze cages. The results obtained are shown in Tables 8 and 9.

As would be expected, cabbages which had been in the solutions 4 days before

the larvae were put on were more toxic than plants which had only been in the solutions 1 day. It can be seen that the relative order of toxicity was

E600 > acetate > oxide > anhydride

TABLE 8. *Third-instar Pieris larvae allowed to feed on cabbages which had been standing with their roots in the insecticide solutions for 24 hr.*

Date: 26 Feb. and 6 Mar. 1952

Material	Concentration (% v/v)	Percentage of foliage eaten on 5th day	Percentage of larvae dead on 5th day
Anhydride	0.2	100	0
	0.1	100	0
Oxide	0.1	100	0
	0.05	100	0
E600	0.01	< 10	100
	0.001	< 10	100
	0.0001	< 10	100
	0.00001	100	0
Acetate	0.05 (w/v)	< 10	10
Control	—	100	0

TABLE 9. *Third-instar Pieris larvae allowed to feed on cabbages which had been standing with their roots in the insecticide solutions for 4 days*

Date: 9 Jan. and 26 Feb. 1952

Material	Concentration (% v/v)	Percentage of foliage eaten on 5th day	Percentage of larvae dead on day	
			5	7
Anhydride	0.2	100	0	0
Oxide	0.1	< 10	50	100
	0.05	< 10	20	90
	0.005	< 10	0	0
E600	0.01	< 10	100	—
	0.001	< 10	100	—
	0.0001	< 10	100	—
	0.00001	100	0	0
Acetate	0.1 (w/v)	< 10	100	—
	0.05	< 10	100	—
	0.005	< 10	20	40
Control	—	100	0	0

This differs from that obtained with *Aphis fabae* Scop. (David & Gardiner, 1951), which was

Acetate > oxide > anhydride = E600.

The cabbage plants in these experiments often showed signs of unhealthiness, clearly due to the transference from soil to solutions, so that critical observations of the phytotoxicity of the compounds could not be made.

(b) *Plants in soil.* As before 20 c.c. of the insecticidal solutions were watered on the soil in which plants were growing, and 4 days later the larvae were placed on the

plants. E600 was again the most toxic compound, but the oxide- and the acetate-treated plants were also poisonous to the larvae. The plants showed no leaf damage due to the insecticides. By the fifth day they were sometimes entirely consumed, but if not the only damage visible was a small amount of nibbling by the larvae.

TABLE 10. *Insecticide solutions watered on to soil in which plants are growing. Third-instar Pieris larvae placed on plants 4 days later*

Material	Quantity present in 20 c.c. solution	Percentage of larvae dead on day		Conditions of plants on 5th day
		2	5	
Anhydride	0.20 (c.c.)	0	0	All eaten
	0.04	0	0	All eaten
Oxide	0.20 (c.c.)	100	—	N
	0.02	0	0	All eaten
E 600	0.01 (c.c.)	100	—	N
	0.002	0	100	N
	0.0002	0	0	N
Acetate	0.1 (g.)	100	—	N
	0.02	100	—	N
	0.002	0	0*	N
Control	—	0	0	All eaten

* Larvae moribund and hardly feeding.

(4) *Systemic action following absorption by the leaves*

In the experiments with eggs and newly hatched larvae described earlier, it was shown that after repeated applications E600 was translocated from the treated outer leaves of a cabbage plant to the egg-bearing untreated inner leaves in insecticidal amounts. Experiments along similar lines have been conducted with third-instar *Pieris* larvae. The results obtained show that E600 and the acetate are translocated in cabbage plants and reach insecticidal concentrations in the young untreated leaves when sufficient quantities are applied to the older untreated leaves (Table 11).

(5) *The relative systemic toxicity of the four compounds to Pieris larvae*

In experiments with *Aphis fabae* on broad beans (David & Gardiner, 1951) approximately known doses of systemic insecticides were introduced rapidly into the plants by absorption through the cut tap roots. This enabled an estimate to be made of the concentration of insecticide in the plant tissue necessary to kill aphids feeding on the sap. An attempt has now been made to obtain the same information for *Pieris* larvae feeding on cabbages. Unfortunately, the cut tap root of a cabbage does not absorb solutions rapidly and neither does the cut stem, as can be judged by the fact that the plants wilt before the insecticide is absorbed. It has been found, however, that sufficiently rapid absorption usually takes place through short lengths of petiole, though some leaves wilt and have to be rejected. Accordingly, the leaves

of young cabbage plants were cut off under water with a razor blade, and the cut petioles were transferred to narrow specimen tubes each holding 1 c.c. of solution. The leaves were placed in a well-illuminated position in a warm greenhouse so as to induce rapid transpiration. If the leaves showed signs of wilting they were given more shade. As soon as almost all of the insecticide solution in any tube had been

TABLE 11. *Systemic action of E600 and the acetate in solutions containing 0.1 % Teepol brushed on to the four outer leaves of young cabbage plants on third-instar Pieris larvae feeding on the inner leaves. One immature inner leaf and the unexpanded crown leaves were left untreated.*

Material	Concen- tration (% v/v)	Treatments		Larvae		Treated leaf damage*
		Total no.	Days on which given	Day on which placed on plant	Effect on	
Acetate	0.5 (w/v)	15	1st to 4th	5th	100 % dead 7th day†	T
	0.1	15	1st to 4th	5th	0 % dead 7th day 75 % dead 10th day	
E 600	0.1	15	1st to 4th	5th	100 % dead 7th day	M
	0.1	1	1st	2nd	Slightly affected 3rd and 5th days	N
	0.05	15	1st to 4th	5th	100 % dead 7th day	M
	0.01	19	1st to 6th	4th	100 % moribund 7th day	T

* Appearance when last treatment given.

† Numbered from day on which the first application of insecticide was made.

absorbed a few drops of water were added. When most of this had also been taken up the leaves were weighed and placed under Petri dishes with weighed larvae. These dishes were ventilated with saturated air to reduce evaporation and prevent the accumulation of toxic vapours. The larvae were allowed 24 hr. to feed, and the remainder of the leaves and the larvae were then weighed. The percentage loss in weight of each leaf represents the evaporation loss plus the amount consumed by the larvae, but it is not possible to apportion the loss between the two at all accurately. However, unless leaves containing insecticide lose water at greater rates than untreated leaves, none of the test leaves should lose more water than a control leaf without a larva, since any part of a test leaf which is eaten cannot lose weight in this way. It may therefore be assumed that if the test leaf loses much more than the 12 % weight lost by the control it is because the larva has been feeding on it. In the case of the larva a gain in weight indicates that over the 24 hr. feeding period the leaf tissue is not sufficiently toxic to prevent the growth of a larva feeding on it, while a loss of weight beyond the average of about 8 % lost by starved larvae indicates a definite toxic action.

The results obtained in this experiment are presented in Table 12. It has been found that larvae gain weight over one day when fed on leaves which have absorbed 2900 mg./kg. (fresh weight) of anhydride and 1020 mg./kg. of oxide. No higher

concentrations were tested. The acetate was very much more toxic, since larvae lost weight when they fed on leaves which had absorbed 50 mg./kg. but not when they ate leaves which had absorbed 6 mg./kg. The E600 was even more poisonous and caused the larvae to lose weight when the leaves had taken up a dose equivalent to about 4.0 mg./kg. At 0.5 mg./kg. of fresh leaf tissue it was apparently tolerated, since the larvae fed and gained weight. Besides being more toxic than the acetate it will be observed that E600 caused a much greater loss of weight in the affected larvae.

TABLE 12. *Showing the approximate dosages of the four insecticides which have been fed to cut cabbage leaves in 1 c.c. of solution and the effect of the leaves on larvae eating them.*

Material fed to leaf	Concentration of solution fed to leaf (% v/v)	Average concentration of material in leaves (mg./kg.)*	Average weight of larva (g.)	Average weight lost by leaf (%)	Average weight lost or gained by larva (%)
Anhydride	0.6	2900	0.117	37	87
	0.4	2400	0.130	38	122
	0.2	1340	0.123	49	125
Oxide	0.2	1020	0.146	43	78
	0.1	550	0.132	30	136
	0.05	280	0.111	35	117
Acetate	0.05 (w/v)	290	0.143	11	-15
	0.01	50	0.145	11	-14
	0.001	6	0.125	26	98
E600	0.001	6	0.188	9	-52
	0.0005	4	0.113	15	-41
	0.0001	0.5	0.135	34	25
Water	0	0	0.118	65	164
Water	0	0	No larva	12	—
No leaf	—	—	0.128	—	-8

* Calculated on fresh weight of leaf.

(6) *The feeding behaviour of Pieris larvae on cabbage plants treated with the insecticides systemically through the roots*

When larvae are allowed to feed on cabbage plants treated systemically with the anhydride up to the maximum concentrations used in these experiments they eat the plant and appear to grow normally. But when compounds which are toxic to the larvae are present in the leaves in rather low concentrations the larvae practically stop eating after nibbling a small quantity of a leaf. Thus they never acquire a rapidly lethal dose by eating a normal amount of leaf containing a low concentration of insecticide. Instead, depending on the toxicity of the leaf, the larvae may either eat enough to grow slowly or they may die after several days, during which they become progressively more shrunken. It must be concluded either that the leaves are unpalatable or that a sublethal dose is acquired which prevents further feeding. When death occurs in such cases it may be due to starvation or to slow poisoning either by the initial dose or this supplemented by occasional subsequent mouthfulls. It has been observed that moribund larvae often recover if placed on untreated

plants. When, on the other hand, a highly insecticidal dose is contained in the plant the larvae may nibble a small quantity of leaf and die shortly afterwards.

There is no doubt that in these experiments the most effective compound, E600, is acting as a stomach poison. Special care was taken to test for a fumigant effect. It was shown that leaves cut from a systemically treated plant were toxic to larvae when there was no E600 solution or treated soil near at hand, and also that larvae remained quite normal when fed on untreated leaves held in close proximity to treated leaves.

DISCUSSION

In the present investigation four compounds previously selected for testing as systemic insecticides against *Aphis fabae* L. (David & Gardiner, 1951) have been tested against the eggs and larval stages of *Pieris brassicae* L. As practical application would almost certainly involve spraying the compounds over the growing brassica crops the tests have included direct contact action, indirect contact coupled with a stomach poison effect (through crawling on and eating sprayed leaves) as well as systemic insecticidal action following translocation from treated to untreated leaves. Although it is not likely to be important for brassica crops in practice systemic action following absorption from the roots has also been studied.

By whichever of the above methods the insecticides are compared they show the same relative toxicity:

E600 > acetate > oxide > anhydride.

Of the four compounds E600 is the only one which is outstandingly toxic to *P. brassicae* eggs and larvae, and it is also the only compound which, contact action excepted, is more toxic to *Pieris* larvae than to aphids as shown by comparison with the figures previously obtained (David & Gardiner, 1951) (Table 13).

TABLE 13. *The approximate amounts of the four compounds required to give complete kills of Aphis adults and nymphs and of Pieris third-instar larvae*

Material	Method of dosing					
	By dipping (% solution)		Systemically from soil (c.c. per pot)		By absorption of measured dose of insecticide (mg./kg. fresh plant tissue)	
	<i>Aphis</i>	<i>Pieris</i>	<i>Aphis</i>	<i>Pieris</i>	<i>Aphis</i>	<i>Pieris</i>
Anhydride	0.05	> 0.2	0.02	> 0.04	50	> 2900
Oxide	0.05	> 0.1	0.002	> 0.02	6	> 1020
Acetate	0.001	> 0.1	0.001	0.02	1	> 6 < 50
E600	0.0005	0.01	> 0.04	0.002	30	> 0.5 < 4.0

E600 also differs from the other compounds in its action on *Pieris* eggs. It cannot be described as an ovicide, since it does not prevent development but kills the larva usually when it is in the early stages of cutting through the egg shell. This action is seen when cabbage leaves bearing eggs are dipped in E600 solutions, and also, which is more interesting, when the E600 is watered on to the soil in which the

plants bearing the eggs are growing. This matter is being investigated in greater detail, since it suggests the possibility of developing systemic ovicides.

It appears from Table 12 that isolated cabbage leaves given a dose of 2900 mg. of anhydride per kg. of fresh leaf and 1020 mg./kg of oxide can be eaten by third-instar *Pieris* larvae. Since the larvae gain weight on this diet it must be assumed that the leaves are not appreciably toxic. Two points should be noted, however. First, since the oxide is volatile, and known to be lost from plants containing it (David & Gardiner, 1951; David, 1952), the total dose absorbed is not likely to be present in the leaf. Secondly, if the toxic action of these compounds depends on prior interaction with some plant constituent, it is possible that at these high dosages there would be insufficient of the hypothetical constituent, probably an enzyme, to render the available quantities of anhydride or oxide toxic. Further, the time during which such a reaction could occur would be rather short.

All the evidence goes to show, however, that although the anhydride may be converted by the plant into an active anticholinesterase which is considered to be the toxic compound (Du Bois, Doull & Coon, 1950), the process is slow in comparison with that which occurs in certain animal and insect tissues (Duspiva, 1951; Hartley, 1951). That effective conversion at these high concentrations might not occur in the plant would therefore be of no importance, especially as at best the concentration of the newly formed toxic compound would only be a small percentage of the parent compound (Hartley, 1951).

It remains to consider the possibility of using any of these compounds as ovicides or larvicides for *P. brassicae*. All the results indicate that the anhydride and oxide are not sufficiently toxic to be used as either conventional or systemic insecticides. That *P. brassicae* larvae are unaffected by feeding on plants toxic to *Brevicoryne brassicae* was noted by Ripper, Greenslade & Hartley (1950). The acetate is not effective as a contact insecticide but is much more toxic as a systemic insecticide applied to the roots than either the anhydride or oxide. A plant dosed with 50 mg. acetate per kg. fresh weight gives a complete kill of larvae, and this concentration compares favourably with that of 50 mg./kg. of the anhydride required to kill *Aphis fabae* on broad beans (David, 1951). When tested as a systemic insecticide applied to the leaves it is found to be translocated and to reach concentrations toxic to larvae in untreated leaves. Its exact potentialities have not been investigated because of its high mammalian toxicity.

E600, diethyl *p*-nitrophenyl phosphate, is very much more toxic than the other three compounds. As a contact insecticide it prevents the emergence of larvae from *Pieris* eggs dipped in 0.005 % v/v solution and third-instar larvae are killed by a momentary immersion in an 0.01 % v/v solution. As a systemic insecticide applied to the soil it is effective against both *Pieris* eggs and third-instar larvae. Thus when 20 c.c. of solution containing 0.001 c.c. of E600 are watered on to 400 g. of moist soil in which a young cabbage plant with 6-8 leaves and about 6-8 in. high is growing, 95 % of the eggs on the leaves fail to hatch and 100 % of larvae which do

hatch are killed. Under similar conditions the solution need only contain 0.002 c.c. to give complete kill of third-instar larvae. These figures compare favourably with 0.02 c.c. of the anhydride required to kill aphids on broad beans following the same test procedure. As a systemic insecticide applied to the leaves E600 is also active. An 0.001 % v/v solution applied to one surface of a cabbage leaf prevents larvae emerging from eggs on the other surface, and with an 0.01 % v/v solution sufficient E600 is translocated from older to younger leaves to have some effect on the emergence of larvae from eggs on the young leaves and to kill a percentage of larvae feeding there. It is to be noted that in all these experiments E600 was brushed on to the treated leaves 8 or more times, but there was also some evidence of an effect in the only experiment in which a single application of 0.1 % E600 was given. E600 is apparently toxic to larvae when present in the plant tissue in concentrations of between 0.5 and 4.0 mg./kg. fresh weight. This is of the same order of toxicity of the acetate (1 mg./kg.) and oxide (6 mg./kg.) to aphids previously found (David & Gardiner, 1951). It may also be seen that E600 is the only one of the compounds which is more toxic to *Pieris* than to *Aphis*, since about 30 mg. E600 per kg. of plant tissue is required to kill aphids (David & Gardiner, 1951).

The mammalian toxicity of E600 is higher than that of parathion (diethyl *p*-nitrophenylthiophosphate) (Schrader, 1947). Unless, however, this, or the persistence of toxic residues, renders it too dangerous for use, it would seem to be worth considering for *Pieris* control. If unacceptable for use on brassica crops it would be a likely candidate for testing against lepidopterous larvae attacking other host plants.

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THE INFLUENCE OF INFECTION WITH *NOSEMA APIS* ON THE ACTIVITIES AND LONGEVITY OF THE WORKER HONEYBEE

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Infection of the adult worker honeybee with *Nosema apis* reduces or obviates brood feeding and causes her to commence foraging earlier than a healthy bee. The length of foraging activity and the total length of life of infected bees is reduced.

In colonies infected with *N. apis* the rate of brood rearing is severely depressed during April, May and June, the degree of depression being proportional to the percentage infection.

Infection decreases during July, August and September, and consequently the rate of brood rearing increases, but the resulting addition in foraging population is usually too late to increase the honey crop.

A worker honeybee normally performs brood-rearing duties in the hive for approximately 18 days and then joins the foraging population. Rösch (1925, 1927, 1930), Nelson (1927) and Haydak (1932) have shown, however, that a bee can adapt her activity to the requirements of the colony. Ribbands (1950) demonstrated that household bees can be caused to forage at an unusually early age, and that bees that have been foraging for pollen can be made to collect nectar instead by anesthetization with carbon dioxide.

Hassanein (1951) noted that infection of the worker honeybee by *Nosema apis* retards or inhibits development of the pharyngeal salivary glands, making it difficult or impossible for infected bees to feed the young larvae. Farrar (1947), in his work on the influence of *Nosema* disease on brood production by recently installed package bees, measured the amount of sealed brood present in each of the new colonies 3 weeks after they had been placed on clean, drawn combs, and attempted to correlate these measurements with the percentage of adult bees infected with *N. apis*. He obtained correlation coefficients of -0.31 in 1945 and -0.42 in 1946. He recorded that newly installed packages of bees having 60–100% of the workers infected with *N. apis* produced in the 3 weeks after installation an average of 100–125 sq.in. less sealed brood than comparable packages of healthy bees.

The experiments described below were made to explore more fully the effects of *Nosema* infection on brood-rearing and on the foraging and longevity of individual bees.

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EFFECTS OF *NOSEMA* INFECTION ON FORAGING AND LONGEVITY

Methods

The bees from a comb of sealed worker brood were allowed to emerge into an incubator. Within 48 hr. each was marked with cellulose paint on the first abdominal tergite using the symbols devised by Ribbands (1949). Fifty bees marked with red symbols were fed individually with syrup containing spores of *N. apis* before being introduced into a colony of which about 30% of the field bees were already suffering from *Nosema* disease. Another fifty bees, marked with blue symbols, were introduced into the same colony and allowed to become infected by contact. A further fifty bees, marked in white, were introduced into a healthy colony in the same apiary as the *Nosema*-infected bees.

The experiments were started in the first week of April 1949 and repeated in August. As soon as the marked bees had been introduced into their respective colonies the entrance to each colony was watched daily for 2 hr., and the identity of each marked bee entering or leaving the hive was recorded. These data made it possible to determine the duration of each bee's foraging activity.

Results

Results are presented in Tables 1-3. Table 1 shows that adult bees infected with *N. apis* start foraging approximately 10 days earlier than normal bees and consequently spend a shorter portion of their lives in performing hive duties. Table 2 demonstrates that the foraging life of the infected bee is considerably shortened, thus diminishing its value to the colony, and Table 3 indicates the effect of *N. apis* infection in reducing the total adult life of the worker bee.

TABLE 1. *Effect of Nosema disease on start of foraging*

	Age at beginning of foraging (days)																							
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24					
April 1949 records																								
No. of bees:																								
Healthy	2	3	7	15	8	6	5	.					
Contagiously infected	2	5	4	2	2	0	2	1	0	6	10	6	5	.	.					
Individually infected	.	3	5	10	12	7	5					
June 1949 records																								
Healthy	2	4	4	14	9	7	5	3					
Contagiously infected	.	.	.	2	4	4	6	3	0	4	0	3	5	9	3	3	.	.	.					
Individually infected	.	.	3	5	9	8	7	6	3					
Aug.-Sept. 1949 records																								
Healthy	2	5	10	18	6	4	.					
Contagiously infected	1	4	5	0	1	1	0	0	2	6	12	10	4	4	1					
Individually infected	.	.	.	6	10	10	10	6	4					

TABLE 2. Frequency distribution showing foraging period of healthy and of infected bees

[illegible]

TABLE 3. Frequency distribution illustrating effect of Nosema disease on longevity

[illegible]

TABLE 4. Degree of infection with *Nosema apis* and number of cells of sealed worker brood (to nearest 100) present in ten colonies of honeybees throughout the season of 1949

Date	Healthy, control, colonies										Diseased colonies									
	A		B		C		D		E		F		G		H		I		J	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
2 Mar.	0	26	0	28	0	27	0	26	0	23	8	20	6	23	10	23	12	24	6	24
10 Mar.	0	38	0	37	0	39	0	40	0	40	10	26	6	27	10	37	12	34	12	33
18 Mar.	0	58	0	54	0	47	0	47	0	48	14	33	12	32	12	39	16	38	14	50
26 Mar.	0	84	0	86	0	68	0	67	0	69	24	36	18	60	16	42	20	49	16	64
3 Apr.	0	108	0	102	0	84	0	90	0	77	24	42	22	64	26	78	30	69	20	76
11 Apr.	0	133	0	122	0	101	0	91	0	91	30	63	32	85	30	84	24	87	24	97
19 Apr.	0	147	0	134	0	110	0	109	0	110	40	66	40	78	30	83	20	108	26	94
26 Apr.	0	145	0	141	0	103	0	103	0	106	48	74	40	76	38	89	38	79	30	90
4 May	0	152	0	149	0	120	0	120	0	115	58	76	54	83	62	79	58	77	40	84
24 May	0	106	0	158	0	125	0	125	0	125	70	80	62	82	50	83	48	83	42	82
31 May	0	134	0	145	0	135	0	125	0	117	60	83	60	86	34	92	40	86	38	85
13 June	0	129	0	131	0	121	0	113	0	114	48	90	44	88	20	97	30	89	20	94
26 June	0	132	0	132	0	117	0	108	0	107	50	88	20	90	10	122	24	101	18	99
9 July	0	111	0	110	0	109	0	107	0	103	28	95	12	103	12	101	10	123	12	103
22 July	0	101	0	103	0	103	0	110	0	98	18	95	12	103	18	99	18	94	8	107
9 Aug.	0	86	0	84	0	84	0	82	0	82	10	89	8	82	20	73	18	76	14	76
25 Aug.	0	71	0	72	0	52	0	61	0	52	8	68	12	67	12	54	20	42	14	45
7 Sept.	0	52	0	63	0	42	0	42	0	44	8	49	12	41	12	35	20	28	12	35
19 Sept.	0	31	0	34	0	24	0	32	0	23	10	24	14	23	14	19	16	21	10	29
2 Oct.	0	10	0	12	0	10	0	12	0	10	8	8	10	10	18	5	16	7	10	10

(a=percentage with *N. apis*, b=no. sealed worker brood cells to nearest 100)

EFFECTS OF *NOSEMA* INFECTION ON BROOD-REARING

A sample of fifty bees was taken weekly throughout the year from each of five *Nosema*-infected colonies and from five healthy colonies in the same apiary. The area of sealed brood in each of ten colonies was measured every 8 days from March to May and thereafter every 13 days. The results obtained, which exhibit some seasonal variation both in the diseased and in the healthy colonies, are shown in Table 4. To eliminate the seasonal effect on the data the amount of sealed brood in the diseased colonies on a given date was divided by the amount in the healthy colonies on the same date. The results, expressed as percentages, are presented in Table 5.

TABLE 5. *Mean amount of sealed brood in five healthy colonies at different times during 1949, together with amount of sealed brood in each of five colonies infected with Nosema apis expressed as percentage of mean amount of brood of the healthy colonies.*

Date	Mean amount brood of 5 healthy colonies (no. of sealed cells)	Amount of brood in diseased colonies				
		Colony F	Colony G	Colony H	Colony I	Colony J
2 Mar.	2606	79	87	90	93	92
10 Mar.	3872	67	70	97	87	84
18 Mar.	5071	66	63	78	76	99
26 Mar.	7466	49	80	56	66	86
3 Apr.	9207	46	70	84	75	88
11 Apr.	10769	59	79	78	81	90
19 Apr.	12214	64	64	68	74	77
26 Apr.	11968	66	63	75	66	76
4 May	13033	59	64	61	59	65
24 May	12606	64	65	65	66	65
31 May	13095	64	65	70	66	65
13 June	12169	75	72	80	73	78
26 June	11903	74	75	102	85	83
9 July	10732	88	96	94	114	96
22 July	10273	93	100	96	92	104
30 July	9362	110	111	94	92	100
9 Aug.	8353	106	98	87	91	91
25 Aug.	6161	111	109	87	69	73
7 Sept.	4862	101	85	72	57	71
19 Sept.	2878	83	79	67	74	102
2 Oct.	1078	77	89	50	68	92

For a colony of honeybees to obtain the maximum crop of honey it is essential that the foraging population should reach its peak at the beginning of the main honey-flow, and should remain near this level throughout the duration of the flow. Any factor checking the development of the foraging population reduces the honey crop.

The serious effect that infection with *N. apis* has on the rate of brood rearing and consequently on the time at which the foraging population of a colony reaches its peak, and also on the height of this peak, is shown in Table 4. This demonstrates that the rate of brood rearing in infected colonies is severely depressed during April, May and June, the months in which it is most important that a colony should build up quickly if its foraging force is to reach its peak in time for the main honey-flow, which in many parts of England commences about the middle of June.

Table 4 also shows that when the percentage of bees infected with *N. apis* drops in July, August and September the rate of brood rearing rises and approaches that of a normal colony. This rise, however, is too late in the season to be of much value for honey production.

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(Received 23 July 1952)

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1952

The Officers and Council of the Association were as follows:

President: R. W. Marsh, M.A.
Vice-Presidents: R. V. Harris, D.Sc.; I. Thomas, M.Sc., Ph.D.
Hon. Treasurer: H. F. Barnes, M.A., Ph.D.
Hon. Gen. Secretary: R. K. S. Wood, B.Sc., Ph.D.
Hon. Programme Secretary: L. Broadbent, B.Sc., Ph.D.
Hon. Editor of the Annals of Applied Biology: R. W. Marsh, M.A.
Hon. Asst. Editor: I. Thomas, M.Sc., Ph.D.
Council: P. W. Brian, M.A., Sc.D.; Prof. J. B. Cragg, M.Sc.; C. E. Foister, B.A., Ph.D.; Miss M. T. Franklin, B.Sc., Ph.D.; R. M. Greenslade, B.Sc., Ph.D.; R. V. Harris, D.Sc.; I. Isaac, B.Sc., Ph.D.; Miss M. A. Keay, M.A., Ph.D.; A. H. McIntosh, B.Sc., Ph.D.; B. D. Moreton, B.Sc.; A. B. P. Page, D.Sc.; I. W. Prentice, M.Sc., Ph.D.

During 1952 fifty-four new members were elected and the following five resignations accepted: Mrs C. D. Dickinson, C. R. Marshall, E. Skillman, J. R. Thomson and P. N. Wilson. On 31 December 1952 membership stood at 642 but the following fifteen Members resigned from the end of the year: L. A. Allen, C. J. Banks, S. A. Barnett, Miss A. G. Erith, T. M. Forrester, J. L. Harrison, Miss L. E. Hawker, H. M. Morris, A. F. Parker Rhodes, K. A. Pyefinch, C. R. Ribbands, K. G. V. Smith, Miss A. W. Speirs, Mrs K. G. Wollacott and Miss G. R. Wykes.

Honorary Membership of the Association was conferred during the year on Dr Hubert Martin.

The Council regret to record the deaths of Dr K. L. Escherich on 22 November 1951 and Dr O. Appel on 10 November 1952, both Honorary Members of the Association, and of Prof. F. T. Brooks on 11 March 1952.

The Council noted with pleasure the conferment of the honour of C.B.E. on Prof. B. T. P. Barker in the New Year Honours, 1952.

Six paper-reading meetings were held in London with an average attendance of seventy members and fifty visitors. A provincial meeting was held in Newcastle on 8 and 9 January, twenty-eight members and fourteen visitors attending. Details of these programmes are as follows.

8 and 9 January. Newcastle Meeting. G. HESLOP-HARRISON: Phenological aspects of egg-hatching mechanisms in *Homoptera*. J. MACLEOD: Notes on inspection methods for interpreting field data. C. ELLENBY: The potato root eelworm cyst as a hatching unit. J. GRAINGER: Recent work on control of potato root eelworm in Ayrshire. K. J. COGHILL: Field observations on Timothy flies (*Amaurosoma* spp.). J. G. HUNTER and O. VERGNANO: Nickel toxicity in plants. Miss J. BAIRD: The effectiveness of native strains of the field bean nodule organism.

8 February. F. C. BAWDEN: The environment in relation to plant virus diseases.

14 March. Symposium on herbicides and systemic fungicides. E. W. SIMON: The relative toxicity of nitrophenols to various organisms. G. W. IVENS: The phytotoxicity of mineral oils and hydrocarbons. E. B. SCRAGG: The effects of hormone herbicides upon cereal crops. R. L. WAIN: Introductory remarks on systemic fungicides. S. H. CROWDY: Techniques in the bio-assay of systemic fungicides. P. W. BRIAN: Antibiotics as systemic

fungicides and bactericides. J. STUBBS: The evaluation of systemic fungicides by means of *Alternaria solani* on tomato. W. G. KEYWORTH: Root injury as a factor in the assessment of chemotherapeutants by the tomato *Fusarium* wilt test.

18 April. H. C. GOUGH: Wheat bulb fly—some problems. D. LEATHERDALE: Correlations of seasonal plant growth and Cynipid gall development. R. HULL: Control of sugar beet yellows in seed crops. M. READ: Establishment of *Rhizobium trifolii* in the field and serological methods of identification. J. E. CROSSE: Studies on the seasonal cycle of cherry bacterial canker (*Pseudomonas mors-prunorum*). J. M. HIRST: A trap for recording short-period changes in atmospheric spore content. R. K. S. WOOD: Impressions of plant pathology in the U.S.A.

3 October. Symposium on forestry problems. J. MACDONALD: Introductory remarks. T. R. PEACE: Some aspects of recent work on forest pathology. M. CROOKE: A brief review of forest entomology in Great Britain. G. D. HOLMES: Propagation problems in forestry. J. D. BLECHLY: The influence of decay in timber on susceptibility to attack by the common furniture beetle, *Anobium punctatum*. R. C. FISHER: New problems in the control of timber insects. J. G. SAVORY: Breakdown of timber by Ascomycetes and Fungi Imperfecti with special reference to conditions in water-cooling towers. W. P. K. FINDLAY: Some observations on dry rot.

12 November. Joint Meeting with Society of Chemical Industry. E. COLLYER: The fruit tree red spider mite and its predators. M. E. SOLOMON: Pest outbreaks induced by spraying. R. M. GREENSLADE: The use of selective insecticides. Prof. G. C. VARLEY: Population theory and economic entomology. E. HOLMES: An agricultural chemist's attitude towards insecticides. J. S. KENNEDY: Biological control. F. H. GARNER: Spraying as seen by the ordinary farmer.

5 December. R. D. WINSLOW: Hatching responses in some *Heterodera* species. C. G. JOHNSON: A reconsideration of the factors operative in aphid migration. T. E. MITTLER: The feeding of aphids. Miss M. GLYNNE: Wheat yield and soil-borne diseases. G. A. SALT: Field experiments on wheat infected by eye-spot. I. F. STOREY: Cauliflower mosaic in Yorkshire. M. J. WAY: Ecology of ants in relation to coconut production in East Africa.

The Provincial Meeting was held in Newcastle on 8 and 9 January, with headquarters in the Department of Agriculture of King's College by kind permission of the Dean of Agriculture, Prof. Wheldon. Members and guests were welcomed on behalf of the Dean by Prof. Pawson, and after tea in the Union attended a paper-reading session in the early evening. They were then entertained to dinner in the Refectory. Further papers were read during the morning of the following day, the meeting ending at lunch-time. The Council wish to record their thanks to Dr Milne and his colleagues for their work in organizing this, the first meeting of its kind since before the war, and for their efforts to ensure its success.

By kind permission of the Directors, the Spring Meeting was held with Pest Control Ltd., Bourn, Cambridge, on 30 May. Some eighty members and guests attended. The meeting took the form of an extended tour by coach of typical fenland farming country with demonstrations of a wide variety of spraying equipment. Those attending were entertained to luncheon and to tea at Mr Bennett's farm at Downham Market. The Council would like to record their thanks to Dr Greenslade and his colleagues for arranging this meeting and to the Directors and Mr Bennett for their hospitality.

The Summer Meeting was held on 10 and 11 June at the Pest Infestation Laboratory, Department of Scientific and Industrial Research, Slough, and the Imperial College Biological Field Station, Sunninghill, at the invitation of the Directors, Mr Herford and Prof. Munro. At both centres parties were conducted around the laboratories and grounds to see the work in progress and there were ample opportunities for discussion of the many interesting lines of work which were exhibited. The Council wish to record their thanks to Mr Herford and Prof. Munro for their kindness in receiving members of the Association, and to Dr Page who was responsible for much of the work entailed in organizing this meeting.

It was decided that a series of special meetings should be held on 13-17 September 1954 to celebrate the fiftieth anniversary of the founding of the Association. A subcommittee of Council has been appointed to deal with the arrangements for this meeting and a Jubilee Fund started following a decision of Council at the last meeting of the year. Details of this fund as it stood on 31 December 1952 will be found in the Treasurer's Report for 1952.

During the year Council decided to prepare a directory of biological laboratories which have come into use since 1945. This is expected to be available for the use of Members towards the end of 1953.

The Association was represented on the Parliamentary and Scientific Committee, the Biological Council and the National Committee for Biology.

The Council wishes to record its thanks to Miss B. M. Stokes for arranging the distribution of copies of *Common Names of Insects and other Pests*.

In conclusion, the Council wish again to thank the Governing Body of Imperial College for their kindness in providing lecture theatres for meetings and in permitting use of their catering facilities.

REPORT OF THE HONORARY TREASURER FOR THE YEAR ENDING 31 DECEMBER 1952

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £2361, an increase of £564 over the sale of the *Annals* in 1951. The income from the sale of back volumes and parts was £306, an increase of £89, while the sale of reprints and advertisements showed a decrease of £93. The cost of production was £3289, a decrease of £490. This was largely accounted for by the drastic decrease in size of the volume. The net cost of the *Annals* therefore was £447 as against £1541 in 1951.

The amount received during the year for subscriptions and entrance fees, including arrears, was £750, a decrease of £14.

Income for the year showed an excess of £201 over expenditure, as against an excess of expenditure of £892 over income in the previous year. This is accounted for by the greatly decreased deficit on the *Annals*.

The Association is once again indebted to the Royal Society for a grant of £200 from the Government grant-in-aid. It also acknowledges with thanks a special grant of £100 from the Sugar Beet Research and Education Committee.

A Jubilee Fund was initiated in December by the allocation of half of two recent bequests given without restriction to the Association for its general benefit.

The net assets of the Association at the end of the year amounted to £2850, an increase of £399. All available surplus funds are invested in National Savings Certificates (£1822) and 2½% Defence Bonds (£400). The estimated value of the stock of the *Annals of Applied Biology* is £189. 18s. 0d.

The improved position of the Association has been achieved by decreasing the volume of the *Annals* to a size below that which is compatible with the present membership of the Association. In order to rectify this and to maintain the present sound financial basis of the Association, the membership subscription ought to be increased by 10 shillings.

REPORT OF THE HONORARY EDITOR FOR 1952

As foreshadowed in the previous Report, it was found necessary, for financial reasons, to issue a smaller volume in 1952 than in 1951. Vol. 39 contained xiv + 626 pages and 23 Plates as compared with xv + 929 pages and 25 Plates in vol. 38. This reduction has unfortunately led to an increase in the period between the acceptance of papers and their publication.

Including papers published in *Proceedings*, the 1952 volume contained 69 papers (18 fewer than in 1951), the subjects being roughly classified as follows: general applied botany, 9; mycology and plant diseases, 15; bacterial plant diseases, 1; viruses and virus diseases, 10; general applied zoology, 1; entomology and insect pests, 9; insecticides and fungicides, 14; nematology, 7; statistics, 1; microbiology, 1. There were 5 papers by non-members, all in *Proceedings*.

The Editor and the Treasurer attended the meeting of editors of biological journals, called by the Biological Council, on 21 November 1952. It was unanimously agreed at this meeting that the advice of the Royal Society should be sought as to the possibility of obtaining increased financial support for the publications of the learned societies in the biological field, either from the Parliamentary grant-in-aid for scientific publications administered by the Royal Society, or directly from the Research Councils of the Government.

Mr M. J. R. Healy was appointed to the Editorial Committee to succeed Mr F. J. Anscombe, whose services, and those of the other members of the Committee, are gratefully recorded.

ASSOCIATION OF APPLIED BIOLOGISTS PLANT PESTS AND DISEASES COMMITTEE, REPORT FOR 1952

Two Meetings have been held during the year, on 7 February and 20 November. K. Holly, A. Ibbotson and E. T. Roberts were appointed by Council to replace H. E. Croxall, N. G. Morgan, and R. S. Pitcher, whose terms of office had expired. At the first meeting J. B. Goodey was elected Chairman and E. Dunn was re-elected Secretary.

Owing to lack of support and difficulty in finding entomologists willing to fill the vacancies on the Sub-Committee appointed for the preparation of a list of definitions of terms used in Economic Entomology, the Committee have recommended to Council that this proposal be discontinued.

Since the existing N.A.A.S. Pest and Disease Assessment scheme largely overlaps the original proposal of the P.P. and D. Committee, namely, the establishment of crop observational plots for the study of natural infestations by pests and diseases, the Committee have agreed that there is little point in continuing with the project. The following resolution, however, was transmitted to Council:

That information on the records of the first date annually of the appearance of pests and diseases was of the greatest importance and that an endeavour should be made to obtain and collect any such information which may be available at Research Stations, College Experimental Farms, Farm Institutes and County Horticultural Institutes.

The Sub-Committee appointed to collect information relating to the susceptibility of fruit varieties to damage by spray materials has prepared a scheme for the collating of the information that exists on the sensitivity of top and soft fruit to lime sulphur sprays. It is hoped to circulate copies of the scheme to entomologists and plant pathologists in the near future.

The Committee considered that in view of the fact that plant pathologists were receiving many plant specimens showing damage caused by herbicides, there was need for the collection and publication of the records of this damage now available at Research Stations and N.A.A.S. centres.

REVIEWS

The Permeability of Natural Membranes. By H. DAVSON and J. F. DANIELLI.
With a foreword by E. NEWTON HARVEY. 2nd ed. Pp. xii + 365. Cambridge
University Press. 1952. 30s.

This book was completed in 1940 and first published in 1943. It is now reprinted without change save that one or two of the more important new references are added at the end of certain of the chapters, sometimes with a brief indication of their contents. The authors have not attempted a thoroughgoing revision, partly because of 'other literary commitments', but principally, they claim, because the 'time is not yet ripe'. There is perhaps some substance in this claim; the exploitation of isotopes for studying the permeability of natural membranes is in full swing and is in process of leading to a drastic revision of our views in many parts of the subject. When the authors do undertake a revision they might usefully devote a chapter to the varying permeability of the arthropod integument, about which very little was known when this book was written. The living cuticle provides many problems in the physiology of controlled permeability; and the dead cuticle illustrates the curious phenomenon of asymmetrical diffusion. A consideration of these matters alongside such classic objects as the red cell membrane, the sheath of the nerve axon, and the skin of the frog would be very valuable to the student of insecticides—and the debt would not all be on the one side.

V. B. WIGGLESWORTH

Guide to the Chemicals used in Crop Protection. By H. MARTIN and J. R. W. MILES.
Pp. 236. Dominion of Canada, Department of Agriculture Science Service.
1952. No price.

Dr Hubert Martin needs no introduction in this country. His work at Long Ashton, his book, *The Scientific Principles of Crop Protection*, and, lastly, his contacts with industry through the Crop Protection Products Approval Scheme to which unselfishly he gave so much time are well known.

In collaboration with J. R. W. Miles his latest publication, a loose-leafed bulletin, is, consequently, of considerable interest. Each product has one to two sides of a sheet devoted to it and it is intended, from time to time, to replace obsolescent or inadequate information by the issue of fresh sheets.

Alternative names, formula, history, manufacture, physical properties, formulation, analysis and biological properties are all briefly considered. Included in the latter is an account of mammalian toxicity, phytotoxicity, compatibility and mode of action. It is perhaps in this section that the biologist may feel a little disappointed as the pests controlled are only very briefly considered. The chemist, on the other hand, will be well satisfied with the large amount of useful information packed into a small compass.

Both biologist and chemist will, however, be only too pleased to have this valuable publication on their desks and be able to thumb the pages thankfully when someone asks, 'What does Methyl phenyl pyrazolyl dimethylcarbamate do?'

DE B. ASHWORTH

Dry Rot and Other Timber Troubles. By W. P. K. FINDLAY. Pp. 267. London: Hutchinson and Co. 1952. 25s.

There can seldom have been a period when the market for books on semi-popular biology was so well served. Most of these books have been beautifully produced and illustrated, many have been well written, but a considerable proportion seem to have been published either to meet a possible market or else merely to continue an established series. Here, however, we have a book which fills a real gap, and fills it with extreme adequacy.

The title is perhaps somewhat deceptive, for the book in fact covers practically everything that can go wrong with timber, during its growth, manufacture and use. The author has taken great pains to ensure that the somewhat difficult technicalities of the subject are made understandable to the general reader, and the first two chapters 'The Nature of Wood', and 'Causes of Deterioration in Timber' are largely introductory. The next three chapters 'Fungal Decay', 'Discolorations, Stains and Blemishes', and 'Destruction of Wood by Insects', cover the main causes of deterioration in timber. The title of chapter vi, 'Diseases of Standing Trees', does not make it clear that, apart from a few introductory remarks, the chapter is concerned solely with decay in the living tree, and the reasons why it occurs.

Chapter vii on 'Care of Timber after Felling and Conversion' is brief, but very much to the point, and is followed by one on 'Wood Preservatives and their Use', which gives a very practical summary of what has now become a vast subject. The last four chapters are concerned with decay of timber in buildings, in farms and gardens, in boats, mines, cooling towers and other specialized sites. The means of avoiding decay are clearly set out, and the text enlivened with very well chosen examples of places where decay has occurred. Of particular interest is the account of the serious decay of wooden naval vessels, before the coming of the ironclads.

This book ends with a very, but I think rightly, brief bibliography, and an index glossary, a combination which might more often be used. It is printed on art paper, so that the numerous photographs and occasional line drawings can appear in their proper context. The photographs are particularly well selected, though in a few places, where two or more appear together on the same page, the dividing line is not clear and one tends to view the whole as a single rather confusing picture.

It is a tribute to the author's style that the book can be read through without boredom or fatigue, and to his accuracy that it can be accepted without any serious reservation. I only found one or two statements that I should have wished altered, and those perhaps were differences of interpretation or opinion, rather than inaccuracies of fact. The book should be of practical use to a very wide public, not only to those concerned with the use of timber, but also to students. It is to be hoped that it will reach, interest and inform an even larger number of people, who may have no immediate reason to use all the good advice given in it.

T. R. PEACE

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1952* Cr.

EXPENDITURE			INCOME		
£	s.	d.	£	s.	d.
To Estimated Value of Stock, 1 January 1952 .					
To Cambridge University Press:					
Paper and Printing	228	0 13 5	By Sales—Current volume	236	1 6 0
Binding	267	7 0	Back volumes, Parts and Sets	306	5 6
Carriage	103	6 7	Reprints and Advertisements	147	6 3
Insurance and Sundries	11	4 0			
Commissions	626	7 4	By Contribution to cost of corrections	2	0 0
			By Estimated value of Stock—31 December 1952	189	18 0
			By Balance carried down	446	14 7
			3288	18	4
			£3453	10	4

GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1952

EXPENDITURE		£	s.	d.	£	s.	d.	INCOME	
To <i>Annals of Applied Biology</i> , balance brought down				7	446	14	7	By Members' Subscriptions:	
To Printing and Stationery				0	45	5	0	Arrears	£ 3 15 0
To Postages and cheque stamps				0	49	0	4	Entrance fees	28 15 0
To Subscription—Parliamentary Science Committee				0				Current	717 18 11
To Donation—Biological Council				0	7	7	0	By Proceeds, List of Common Names:	750 8 11
To Sundry out-of-pocket expenses of Editors, Secretaries and Treasurer				0	1	10	0	Part 1	
To Refreshments				6	11	4	6	Part 2	13 15 9
To Hire of Rooms				9	22	12	9		36 5 4
To Audit Fee				6	4	14	6	By Interest received and receivable:	
To Printing List of Common Names:				0	7	7	0	2½% Defence Bonds	10 0 0
Part 1. Corrigenda				0				National Savings Certificates	47 10 0
Part 2				6	78	17	6	Post Office Savings Bank	20 11 6
To Balance being excess of Income over expenditure for the year					81	12	6		78 1 6
					201	3	4		
					£878	11	6		£878 11 6

BALANCE SHEET AT 31 DECEMBER 1952

Cr.

Dr.

LIABILITIES AND SURPLUS			ASSETS		
	£	s. d.	£	s. d.	£
Sundry Creditors and Accrued charges:					
Cambridge University Press	574	0 7			498 14 7
Audit Fee	7	7 0			744 8 9
Subscriptions and Entrance Fees paid in advance			581	7 7	
Life Membership Fund Reserve:			23	13 4	
As at 1 January, 1952	175	0 0			1821 13 9
Addition during year	25	0 0			400 0 0
Excess of Assets over Liabilities:			200	0 0	189 18 0
As at 1 January, 1952	2451	0 10			3654 15 1
Add: Royal Society Grant	200	0 0			110 18 0
Special Grant	100	0 0			
Excess of Income over Expenditure for the year 1952	201	3 4			
Less: Transfer to Jubilee Fund	2952	4 2			
	102	10 0	2849	14 2	
			3654	15 1	
			110	18 0	
			£3765	13 1	
Jubilee Fund					£3765 13 1

H. F. BARNES, Hon. Treasurer

I certify that the foregoing accounts are properly drawn up in accordance with the books, vouchers (Signed)

J. B. BENNETT } Auditor
Chartered Accountant }

HARPENDEN, 24 March 1953

Vol. 40, No. 3

September 1953

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

F. C. BAWDEN

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MR R. W. MARSH, M.A.

President of the Association of Applied Biologists, 1951-53

THE PAST AND THE FUTURE OF
THE ANNALS OF APPLIED BIOLOGY

By R. W. MARSH, M.A.

*Long Ashton Research Station, Bristol*ADDRESS OF THE PRESIDENT OF THE ASSOCIATION OF APPLIED BIOLOGISTS,
DELIVERED TO THE ANNUAL GENERAL MEETING ON FRIDAY, 17 APRIL 1953

By the time that this address is in print the Association will be within a few months of celebrating its Golden Jubilee. This is therefore an appropriate time to attempt to link a prospect of the future of *The Annals of Applied Biology* with a glance at its past. It was an encouragement in undertaking this task to know that much of the spade work had already been done by Prof. Brierley's history of the first twenty-five volumes, published on pp. 186-192 of volume 26—a paper that should be 'required reading' for every member of our Association.

Prof. Brierley records that when the Association of Economic Biologists was inaugurated in 1904 the only form of publication adopted was an annual statement of *Proceedings* containing Council Reports, notices of meetings and abstracts of papers read. Four such *Proceedings* were published separately for the years 1905 to 1908. In the meantime, that pioneer economic entomologist, Mr W. F. Collinge, then Honorary Secretary of the Association, founded, as a private venture, *The Journal of Economic Biology*, first published 15 November 1905. This *Journal* came to be recognized as the unofficial organ of the Association, and in 1908 the connexion was made closer by an arrangement with Mr Collinge, whereby he agreed to supply the *Journal* to Members of the Association. From 1909 to 1912 the Association's *Proceedings* were included in the *Journal*.

The financial and general control of *The Journal of Economic Biology* remained in the hands of its proprietor, Mr Collinge, and by 1913 it had become clearly unsatisfactory that the Association's publications should be issued in a journal over which the Association possessed no editorial supervision. At a Special General Meeting held in September 1913 it was decided that the arrangement with Mr Collinge should be allowed to lapse at the end of that year and that the Association should publish a journal of its own.

Prof. Brierley reminds us that at the time that this decision was taken the Association had a membership of 105: its receipts for 1913 amounted to £66. 12s. and its expenditure to £88. 13s., while its total reserve was £100. Nothing daunted, the leaders of the Association of forty years ago, notably Prof. Maxwell Lefroy, took the risk and, in May 1914, part 1, volume 1 of *The Annals of Applied Biology* appeared.

After volumes 1 and 2 had been published, Prof. Lefroy resigned the editorship

and was succeeded by Mr E. E. Green, who was Editor from 1916 to 1921. In the war years of 1914-18 the finances of the Association were severely strained, but publication was maintained with the aid of two grants of £50 from the Royal Society. In 1919 and 1920 the financial position of the Association temporarily improved but the demand for space increased and—to use an expression that has a familiar ring—the expense of producing a larger *Annals* caused disquiet in the mind of the Honorary Treasurer. An application was made to the Development Commissioners, who took the unprecedented step of granting a sum of £200 in aid of publication. For this, the Association was greatly indebted to the influence of its then Treasurer (the late Sir John Fryer). The succeeding volume of the *Annals*, volume 7, was an opulent production with twenty-six plates, three being in lithograph and one in colour. The Council decided that a return to austerity was imperative.

A change of editorship followed, and in 1921, Prof. Brierley and Mr Ward Cutler undertook the task of surmounting the crisis. At the same time the annual subscription was raised from 20s. to 25s. and the price of the *Annals* to non-members became £2 per volume.

From 1921 to 1939 Prof. Brierley piloted the *Annals* through a period in which it increased steadily in size and rapidly in reputation. Mr Ward Cutler was succeeded as Zoological Editor by Prof. J. W. Munro in 1933, and by Mr C. T. Gimingham in 1934. From 1925, four numbers a year have been published in unbroken succession. Volume 24 (1937), of 940 pages with 51 plates marked the peak of this period of stability.

Following the outbreak of war in 1939, the first problem that arose was not one of finance but of shortage of paper. The supply allotted to the Cambridge University Press for scientific journals was reduced to one-third of the pre-war figure and this brought about a situation in which the possibility of suspending publication was seriously considered. It so happened, however, that the office of the Paper Controller was situated in Reading, a fact of which Prof. Brierley took the fullest advantage.

In 1940, the *Annals* was, in part, printed in smaller type, and by 1941 the volume had shrunk to 416 pages and the quality of the paper had much deteriorated. The influx of scripts, however, showed no diminution and, in 1943, with many misgivings, a two-column format using smaller type was adopted throughout. As the war continued, the depletion of the Cambridge University Press staff and, in particular, the shortage of skilled compositors, slowed up production and led to increasing delays in the issue of the quarterly parts.

Every member of the Association, and in fact every user of the *Annals*, owes a profound debt of gratitude to Prof. Brierley, whose indomitable resolution so far overcame difficulties that at the end of the war it could be recorded that the *Annals*, almost alone among British scientific quarterlies, had maintained its four numbers a year in unbroken sequence. As Mr Gimingham wrote in 1946:

When Professor Brierley took over the editorship, the affairs of the Association were somewhat under a cloud and the possibility of continued publication of the *Annals* was in doubt. Since then, the *Annals* has gone from strength to strength and now has a high reputation, both at home and abroad, as a leading biological journal. . . . The editor has been generous of his time and energy, always with the aim of ensuring a high standard, both from the scientific and literary points of view, for the papers published; no trouble was too great for him to take and no detail too small for careful consideration.

May I record here, as a postscript to this tribute, that Mr Gimingham himself, as joint editor from 1934 to 1944, played his full part in this crucial period of *Annals* history.

From 1945 onwards the paper shortage eased and in that year the number of copies printed was increased from 700 to 800. In 1946 Council sanctioned an expansion of the volume to 465 pages and the printing order rose to 1000, then to 1200 in 1947 and 1450 in 1948. The shortage of skilled compositors remained a limiting factor, and, throughout the late 1940's the numbers never appeared punctually, the delay being sometimes as much as three months. Volume 34 in 1947 returned to the single-column format and 11-point type: this number was of 650 pages, a size that remained fairly steady up to 1950. For 1951, Council authorized a determined attempt to meet authors' complaints of delay, this delay arising from the fact that the rate of intake of papers was exceeding the rate of publication. The result was the issue of volume 38 with 929 pages and 25 plates: this had the effect of reducing the time between acceptance of a script and its publication to approximately eight months.

In the same year the printing order was raised to 1700 copies, indicating that the circulation of the *Annals* in 1951 was approximately two and a half times that in 1945.

By this time the successive increases in printing costs had reached what we hope will prove a peak, and in the year in which volume 38 appeared the Association's reserves fell by nearly £900. Last year (1952) saw a return to a 600-page volume but unfortunately also a return to a twelve months' delay for the authors. This is the point at which it seems appropriate to leave the historical record and turn to the possibly less arid topic of the policy of the *Annals*.

FUNCTION OF THE *ANNALS*

A consideration of the policy of the *Annals* raises numerous problems common to a wide range of scientific journals. It may seem unnecessary to defend the existence of scientific journals in general, but, as many of you will remember, their essentiality was called into question shortly before the Royal Society's Scientific Information Conference of 1948. It was then suggested that, ordinarily, papers should be issued only as separates, which would be edited and distributed by a National Distributing Authority. Only selected papers, chosen for their outstanding permanent value, were to be collected and bound for the use of reference libraries.

Preliminary canvassing of this suggestion showed that it was unrealistic. One of the facts established was that those who habitually consulted scientific papers looked

for about three-quarters of them in the pages of bound journals, and only 11 % in collections of reprints. From the many reasoned defences of the existing method of publication, I quote one by Sir Alfred Egerton, who said:

The scientific journals are the outlet of the scientific investigator, wherein he can record his observations and achievements; not only this, for they impel him to clarify and express his ideas and to discuss the results of his work in relation to other work and to maintain a high standard. Communications for publication in scientific journals are subject to editorial requirements and to referees before publication, often to discussion at meetings, and are then open to the criticism of all readers. The whole procedure is an essential feature of science.

This view was so obviously held by almost everyone taking part in the 1948 Conference that the proposals for the virtual abolition of the present system of publication were withdrawn before the Conference formally opened.

The Conference, being satisfied that scientific journals justified their existence, set up a number of Working Parties, one of which was asked to report on the grouping of scientific communications within existing journals and to comment on the functions performed by journals publishing papers covering a wide field of subjects. A number of memoranda and reports (now published in the *Report of the Scientific Information Conference**) were carefully considered and the conclusion was reached that it was desirable to retain a certain flexibility in the grouping of scientific publications for the following reasons:

- (i) rigid dividing lines between the sciences cannot be set up, and the centre of importance is continually changing;
- (ii) journals and societies at times come under the control of individuals or groups of individuals who do not adequately represent their branch of science, and in these cases the existence of alternative channels for publication provides a powerful means of securing reforms;
- (iii) an author whose paper is rejected by one journal should have at least one chance of securing its acceptance elsewhere;
- (iv) certain journals belong to societies who cater for their members or Fellows who may . . . cover a wide field.

I should like to take up the point raised under (iv), since this aspect links up so closely with the words used by Prof. Maxwell Lefroy in the Editorial of the first number of *The Annals of Applied Biology*. Prof. Lefroy wrote:

The Association . . . commences herewith the publication of a journal devoted to the special interests of its members . . . *The Annals of Applied Biology* is intended to cover the ground in applied biology which is not now covered by special journals such as those dealing with agricultural science, parasitology, genetics and medical science . . . All papers which bear on the scientific problems of applied biology will be welcome; we have no place for purely systematic work which is amply provided for elsewhere, nor for faunistic work as such.

Prof. Lefroy also expressed the hope that the publication of the journal would have a great influence in forming a link between the workers in Great Britain, the Dominions and the Colonies.

It is not easy to provide a conspectus of the fields that the *Annals* has, in fact, covered. We have, however, Prof. Brierley's classification of the 960 papers received

* Short extracts from this *Report* are reprinted by permission of the Royal Society.

from 1914 to 1938 and I have given below, in column *A*, the figures in each of Prof. Brierley's groups expressed as percentages.

	<i>A</i> (%)	<i>B</i> (%)
General applied botany	14	15
Mycology and fungus diseases	17	18
Bacterial diseases	5	1
Viruses and virus diseases	11	13
General applied zoology	3	5
Entomology and insect pests	26	19
Insecticides and fungicides	10	18
Nematology	2	5
Microbiology	7	2

If we apply this same classification to the 860 papers published in the years 1939-52 the result is as listed in column *B*.

There is, I think you will agree, a remarkable degree of similarity between these two columns of figures. This might be an indication that the *Annals* in its pre-war years evolved a pattern that succeeded because it corresponded to the needs of the Association and to those of its outside readers.

In drawing up such a table, however, one encounters repeated difficulties in placing papers in the set categories. Looking back over the contents lists of the *Annals* volumes, the titles most frequently recurring are seen to be those that refer to interrelationships between plants, viruses and insects, to toxicity studies of chemical materials against insects, fungi and plants, to soil microbiological studies and to investigations of a wide range of environmental factors affecting plant or insect physiology. These are problems of which the solution can come only from the co-operation of workers in widely separate scientific fields. The interplay of inspiration between chemists and biologists, between plant physiologists and statisticians, between mycologists and soil scientists—these, and an infinite number of like permutations within groups of research workers provided the advances for which the *Annals* is a natural channel of communication. Such work bridges the divisions between the conventionally labelled 'subjects' in biological work and may start symbiotic growths capable of developing into new 'subjects' of great potentialities.

There has probably always been in the Association two drifts of opinion corresponding to those tendencies that divide systematists into 'lumpers' and 'splitters'. Up to 1944 the Association showed a well-marked dichotomy between its botanical and zoological interests: this was reflected in the *Annals* by the existence of a botanical editor and a zoological editor, each of whom received papers independently. In 1944 Council recorded that: 'in view of the increasingly wide range of subjects now covered by the Association, many of which cannot be classified as strictly botanical or zoological. . . the Council have decided that the time has come to cease dividing the Association into botanical and zoological sections.' Concurrently it was agreed that all manuscripts should be first submitted to a General

Editor and then allotted for refereeing to a Publications Committee, a body to which I shall refer later.

In 1942 the President of the British Mycological Society suggested that the Plant Pathology Committee of his Society should merge with the Association of Applied Biologists to form an association of all those interested in plant pathology in its widest sense. The corollary to this proposal was a change from an *Annals of Applied Biology* to an *Annals of Plant Pathology*.

Since this proposal was mooted, the Plant Pathology Committee have shown no disposition to adopt it, while we have seen in the *Annals* an increase in the representation of subjects (such as studies of animal pests and of stored-products infestation) that make a valuable contribution to the *Annals*, but could not be included in a journal of plant pathology.

More recently, there have been suggestions for regional sections within the Association and for splitting the *Annals* in various ways. The time may come when a branch of the *Annals* may split off under its own weight, but I cannot envisage the retention of the prestige of the *Annals* by any offshoot publication. But I should chiefly regret any such splitting because it would be one more step in the direction of disunity.

In each field of biology and in more and more divisions of these fields there is the need for the specialist journal. But as division increases, so does the need increase for bridging and integration. The *Annals*, as the organ of the Association of Applied Biologists, would fail in its purpose if it ceased to reflect the diverse interests as well as the links between all Members of the Association. We can state it as an axiom that any association of biologists will be a highly fissionable body. It is the more important that any force making for unity in this body should be maintained.

In expressing the conviction that the *Annals* should retain its character as a multi-purpose journal, I do not imply that space should be found for papers on a wide variety of topics, irrespective of the merits of the contributions. Circumstances to-day demand a most rigorous selection. Twenty years ago there was no major difficulty in finding room for all papers reporting new work in applied biology. Since then there has been a most rapid expansion in staffs, facilities and expenditure in this field. Taking, as a sample, the *List of Agricultural Workers in the British Empire*, the 1931 list contained approximately 2500 names. The corresponding list for 1950 included 8000 names. Here there is a three-fold increase, and we may safely assume that the increase in University staffs and in industrial appointments concerned with biology is at least of that order. Further, the staffs to-day have the benefit of far more technical assistance and of more ample laboratory and field facilities than in 1930. The output of publications, therefore, is likely to have risen even more steeply than the number of research workers.

The means employed to meet this situation in the biological field have been, first, the founding of new journals. Examples from the United Kingdom that will readily occur to you are *The Empire Journal of Experimental Agriculture* (1933),

The Journal of General Microbiology (1947), *The Journal of Soil Science* (1949), *The Journal of the Science of Food and Agriculture* (1950) and *Plant Pathology* (1952). In addition, there are ever-increasing numbers of monographs, bulletins, proceedings of symposia, reports of research institutes, technical communications of the Commonwealth bureaux, publications of the Research Councils and the D.S.I.R. There is, too, a growing tendency to publish results of original work in privately owned periodicals such as *Nature*, *Research* and *Science*.

To some extent, as indicated above, government-aided work is being issued in state-financed publications. This helps to reduce the pressure on the journals maintained by the publishing societies, but has its limitations. A government publication might be unable to include papers from outside sources, and the contributions published might not have the benefit of criticism by an independent editorial board.

The existence of additional channels for the publication of papers in applied biology and cognate subjects does enable the *Annals* to be more selective in that it can give preference to contributions that provide information of value outside, as well as within the field of work with which the paper is primarily concerned. Further, it occasionally provides the opportunity for the allocation of a script to an obviously more appropriate journal. This topic was considered at the Scientific Information Conference, and it was recommended that such activities should be furthered by setting up standing committees of editors, the function of these committees being consultative, not directive. At present, no such consultations take place, mainly because of geographical difficulties. If, however, we can look forward to the re-appearance of the plan for a Science Centre, discussions of this type may become practicable. This is a topic to which I shall refer later.

To return to hard facts, it is evident that the extending channels of publication in applied biology and the bordering subjects have barely kept pace with the increasing flow of papers. In such circumstances the Editorial Committee has every incentive to maintain a high standard, but even so, the rate of acceptance of papers continues to outstrip the rate at which they can be published. The inevitable result is delay.

From the author's point of view, any delay in publication after the paper has been completed is a source of irritation, but there will always remain an irreducible minimum of delay between the receipt of a typescript and its issue in a completed number of the *Annals*. The University Press require a period of four months for preparation of copy, composition, making-up, corrections of first proofs, corrections of revise proofs, printing, binding and distribution. The circulation of typescripts to members of the Editorial Committee and, sometimes, to additional referees, may take one or two months. A period of six months therefore remains as unavoidable delay, whatever measures may be taken within our present system of conducting the *Annals*. When, in 1951, by publishing a 900-page volume, delay was reduced to eight months, the minimum was very nearly achieved.

Of recent years, as you know, the limiting factor in determining the size of the *Annals* has been the financial resources of the Association, a topic to which I shall have to refer. But even if we could find more space in the *Annals* I am convinced that it is in the best interests of both authors and readers that there shall be no relaxation of the efforts to remove all unnecessary bulk in the individual papers.

PRESENTATION OF PAPERS

For many of those now becoming authors, the composition of concise papers has been made a matter of special difficulty by the experiences of their early graduate years. What may be called the apprenticeship stage in the art of authorship comes often in the composition of a thesis or in the writing of annual reports for the Director of an Institute or for a Research Council committee. A statement often found in University Calendars is that theses must be presented in a state suitable for publication. It would seem a somewhat broad interpretation of that requirement that encourages theses of 50,000 words or more accompanied by tables, diagrams and photographs to be numbered in hundreds. Such theses are, in fact, the record of every possible detail of the candidate's work. Again, the reports made to heads of departments or to grant-allotting bodies are often statements of how the writer has spent the period since he made his previous statement. In both these examples, inclusiveness is accounted a merit.

Now if we turn to a contribution in a scientific journal and consider it from the viewpoint of readers, what do we look for? We hope to find a fact proved, an apparatus described, a controversy settled. I do not propose to add to the many admirable publications that advise on the writing of scientific papers, but may I make one suggestion to the commencing author? At an early stage in the composition of the paper, write the summary. Write it with care, because it will have far more readers than the text. The summary will go into the abstracting journals. From there it will become a paragraph in the next annual review of the subject under discussion. In its final stage it will become a sentence or two, or perhaps only a few words, in the new edition of the standard text-book in that field. So, perfect the summary.

Anyone who is induced by a synopsis or reference to read the full text will expect to find this text mainly concerned with the facts summarized. Concentrate therefore in the text on the points strictly relevant to the main theme. It should not be part of the reader's task to discard the superfluous fat before finding the meat that he can assimilate. It is not the function of a scientific journal to produce opulent and detailed accounts of any worker's activities.

I am indebted to Prof. Brierley for the quotation: 'A writing is not perfect when nothing more can be added, but when nothing more can be taken away' (Antoine de Saint Exupéry).

The change from the thesis style to the scientific-journal style requires, in fact, an almost complete reversal of approach, from the inclusive to the exclusive.

Understandably this change is difficult, and the difficulties, for authors, readers and editors, could be lessened if, in the first place, Universities would attach more importance to conciseness and clearness of presentation of theses. A relatively small number of external examiners deals with all the theses in biological subjects presented in this country, and, if they united in demanding conciseness, this modern growth of the bloated thesis could rapidly be deflated.

Secondly, it is apparent that while many directors and heads of departments help their staffs by constructive criticism of their manuscripts, others allow scripts from their scientific officers to be sent to journals without taking any steps to see that these scripts are fit for submission. A recommendation from the Scientific Information Conference can appropriately be quoted here:

The heads of departments in the universities and technical colleges and the directors of Government laboratories, research associations and industrial laboratories might be urged through their associations and by personal letter to do their utmost to ensure:

- (i) that MSS. or TSS. submitted are written in clear and concise English and are prepared in accordance with the rules of the journal to which they are submitted;
- (ii) that to the best of their knowledge and belief a contribution passing through their hands, whether as communicator or as referee, is worthy of publication on its merits as scientific information rather than primarily for 'economic reasons';
- (iii) that they regard it as a duty to the scientific community and their own organization to help their students and assistants to submit only material which conforms to (i) and (ii) above.

I shall make only a passing reference to illustrations and tables. The provision of plates with authors' scripts shows, I suspect, some correlation with the provision of photographic facilities at the institute where the work is carried out. Many of these illustrations are decorative rather than functional. The criterion must be whether the illustration is indispensable to the understanding of the paper. Text-figures can often be more informative than plates, and I suggest that Mr Staniland's work on mechanical aids to the production of text-figures deserves wide attention.

Concerning tables, each paper needs a law unto itself. Broadly speaking, a text too thickly clotted with tables conveys the impression that the data have mastered the author, rather than vice versa. As with the text, the reader will select if the author does not, and it is preferable that the decision on which tables are essential shall be the author's.

A saying attributed to Faraday is: 'There are three necessary stages of useful research—the first to begin it, the second to end it, and the third to publish it.' A consideration of Faraday's second stage leads naturally to the topic of series of papers, i.e. those having a common title followed by a numeral and then a subtitle. (In passing, may I say that these complex titles are the bugbear of the indexer.)

Clearly there can be no question of the values of the series that have appeared in the *Annals* in the past. The scope of a series should, however, be clearly envisaged from the outset, and the end should be at least in sight when the writing-up begins.

Otherwise there may be a tendency to send in the first year's harvest of results as no. 1 of a series, followed by succeeding crops becoming annually thinner as that particular field of study becomes worked out.

FINANCIAL ASPECTS

I have dwelt rather heavily on what might be called the repressive measures associated with the publication policy of the *Annals* because it is necessary to establish incontrovertibly that the factors contributing to the increasing expense of the *Annals* do not include any distension of the volumes arising from an easing of the restrictions governing acceptance or presentation of papers. As authors well know, texts are ruthlessly pruned, illustrations and tables deleted and alternative channels of publication suggested, but there is still a steady growth in the annual volume of material that the Editorial Board considers that the *Annals* should publish. This situation, coinciding with a steep rise in printing costs, has had financial effects that could far more clearly and appropriately be described by the Treasurer. The essential facts are that a volume of the *Annals* now costs around £3250, compared with £1250 in 1939, but this is to some extent offset by the fact that sales to non-members, which totalled £860 in 1939, are now around £2400, of £2800 if we include sales of back volumes. If we have reached the peak of printing costs and can hope that the reputation of the *Annals* will continue to attract more outside sales, it is possible to look ahead to a balanced budget on the basis of a volume of 750–800 pages. In the meantime the burden of the deficit is being borne by 650 members as compared with the 330 of 1939.

By way of recompense, the members receive the *Annals* at much below its economic price and—to put the matter in its most sordid aspect—the possession of a run of *Annals* numbers is not a wasting asset. Further, the *Annals* is the only link between *all* the members and is a major factor in maintaining one of the special characters of our Association: the fact that it unites, on an equal footing, research and advisory workers in both public and private service, both home and overseas. Those who pay for a journal are likely to read it, either carefully or cursorily, and consequently members secure that opportunity of maintaining contact with other fields of biology, which I have already claimed as a *raison d'être* for the *Annals*. It has been suggested that a membership at reduced fees should be instituted for members of the Association who do not wish to receive the *Annals*. This suggestion would become ripe for consideration only when the Association reaches the point when a drop in revenue could be accepted with equanimity. I have indicated that such a time may come, but it is obviously some years ahead.

There must be, however, a ceiling to the subsidizing by the research worker of scientific journals—a tax that falls particularly heavily on those who wish to remain members of a number of publishing societies. Our Association shares with many other bodies the problems that were so ably stated by Dr Ainsworth in his letter to *The Times* published on 19 March 1953. As the financing of our journal is supported

by members' subscriptions, the increase in publication necessitated by the expansion of the scientific services becomes a charge on the individual workers. In effect, we have to pay for the publication of our research.

These matters were recently discussed by a number of publishing societies whose representatives were called together by the Biological Council on 21 November 1952. It was shown at this meeting that the measures already being taken to deal with the difficulties of the publishing societies are by no means negligible. The Royal Society has long been active in representing the interests of these societies and in administering grants-in-aid from the Treasury which in 1952 totalled £25,000. From this fund, the Association has been helped for many years, and it is fitting that we should take this opportunity of expressing our gratitude to the Royal Society for their continuing interest and help. It was, however, pointed out at the Biological Council meeting that only one-tenth of the total grant-in-aid went to biology and this was shared among fourteen societies. The major publication grants are allotted to the journals concerned with the physical sciences. Government expenditure on research in biology normally makes no provision for the publication of results in scientific journals. In 1952 the grants made to the Agricultural Research Council, the Medical Research Council and the Department of Scientific and Industrial Research totalled approximately £10,000,000. If one-tenth of 1% of these research grants was allocated to the publishing journals in the biological field, the financial problems of those journals would be solved.

There would be obvious advantages in attaching such funds to the existing grant-in-aid for publication and leaving their administration in the hands of the Royal Society.

Another matter raised at the Biological Council's conference was the possibility of effecting economies in printing costs. Note was taken of the fact that printing could be done at relatively cheap rates by local presses. On the other hand, it was agreed that the University Presses maintained a remarkably high standard of production and accuracy and, in effect, assisted editors by preparing copy and by providing proof-reading of almost uncanny proficiency. I cannot resist paying a personal tribute to the press reader who scrutinizes the proofs for the *Annals*. He it is who unearths the discrepancy between some value hidden away in a table and a statement in the text some pages away. He is the man who discloses that an author's spelling of (say) 'nematicide' is not that used by the same author three years previously. Such service can be provided by very few Presses and any change to a less experienced printer would necessitate one to a more skilful and leisured editor.

Editorial work has two aspects: the first is the critical aspect—the decision whether a script is to be accepted as it stands, sent to referees, submitted elsewhere, or rejected. In the *Annals* much of this work falls on the editorial board. The other side of editorial activities includes matters of routine—maintaining communication between authors and publishers, collecting and distributing proofs, dealing with requests for reproduction of illustrations, arranging for book reviews and so on.

The first aspect must always, in my view, remain the function of unpaid appointed representatives of the publishing society. The second aspect—the ‘post-office’ activities—could be more efficiently handled by a paid editor who, preferably, would do this work for a group of societies of allied interests. To this limited extent, I am in favour of the rationalization of publication of scientific journals. I cannot however envisage such a development until the plans for a Science Centre materialize.

A joint editorial office in the future Science Centre, giving the *Annals* a permanent headquarters would lead to other benefits. For thirty years the Editor has been sent three reprints of every paper published in the *Annals*. The remains of the pre-1945 collection are at present at Reading: the rest are at Long Ashton where they provide a storage problem and are virtually inaccessible to Members. Similarly, the stream of books, journals and reports, sent to the Editor for the benefit of the Association, now fail to achieve that purpose.

I repeat, however, that whatever re-planning of the editorship may come, it is vital that the principle and functions of an editorial board shall remain. Reverting for a moment to the history of our journal, we find that the Editor in its early years had the assistance of a committee of eight or nine outstanding biologists. In 1919, Law XIV of the Association stated that the Council should appoint from the Members of the Association an Editorial Committee who should be responsible for the publications. Three years later the law was made more precise and worded as follows: ‘The Council shall appoint a Publications Committee consisting of the Editors, the Treasurer, two ordinary Members of the Council and two ordinary Members of the Association.’ Seven years later the following words were added: ‘who shall be responsible for the Journal of the Association’. Finally in 1949 the present Law XIV was adopted, which reads: ‘The Council shall appoint an Editorial Committee consisting of not more than four members to assist the Editors in the publication of the Journal of the Association.’

I am not able to speak with any real knowledge of the work of the Publications Committees of pre-war days but when I first joined the Committee in 1942 I did not find that the duties were onerous. That situation is now completely altered. The 1949 change in the Law permits more continuity of service on the Committee, whose members now take a burdensome but decisive share in conducting the *Annals* and maintaining its standards. An appendix to this address sets out in detail a table of the Committee members since 1938: I must confine my present comments to those whose service has been outstanding.

First, Dr Ieuan Thomas, who came on to the Committee in 1944 and, since 1946, has been Assistant Editor. It would be more correct to describe him as Joint Editor, since all that major section of the *Annals* pertaining to insects and other less important animals is his responsibility. Also, as you know, he carried all the editorial duties single-handed in 1949 and 1950. Dr F. R. Petherbridge served on the Committee throughout the difficult years 1940 to 1944: Dr Hubert Martin

helped us with his unrivalled knowledge of insecticides and fungicides for eight years, in 1940-1 and 1945-50. Mr D. J. Finney came to our rescue in 1946-50 at a time when the impact of statistics on biology was presenting entirely novel problems in presentation. Dr D. J. Watson has now for four years provided constructive criticism of the papers concerned with plant physiology. Nor should we forget the many referees outside the Committee who have given unstinted help. But on the Committee itself I have still to mention our senior member. Mr Bawden's name has graced the front cover of the *Annals* since 1943, and I welcome this opportunity of affirming that his judgement, his discrimination, his insight, have been determining factors in ensuring that prestige in which the *Annals* is now held throughout the world.

May I close on a personal note of sincere acknowledgement of the help I have had from the Editorial Committee, from the Officers and Council (particularly from the Treasurer), and, not least, from the authors of papers. An author's paper is his brain-child and no one cares to see his children maltreated. The exigencies of these times make it inevitable that some contributions receive unnecessarily rough handling. My fellow-editor and myself, and the members of the Editorial Committee would consider it understandable if we sometimes received letters echoing a note of resentment. Not every author, I admit, writes to me (as one did) saying that he found the task of cutting his paper by 50% 'positively exhilarating'. But I have never received any letter that did not show a generous forbearance, a readiness to appreciate present difficulties and to accept the restrictions directed to making the *Annals* of the greatest service to the greatest number.

In conclusion, we can feel that after the vicissitudes of forty years the *Annals* has become something in the nature of a trust handed down to present members of the Association. It has developed its unique pattern by a weaving together of the varied threads found in our Society. That, in my view, is a salient contribution made by the *Annals* to the advancement of scientific knowledge, and forms the aspect of its character that we should most zealously preserve. As long as the *Annals* continues to exist as the palpable symbol of the unity of the Association, so long will its future prospects gleam as brightly as do its past achievements.

APPENDIX

The Annals of Applied Biology *Editorial Committee, 1939 to 1952*

Year	Editor	Assistant Editor	Other Members of the Committee
1939	W. B. Brierley	C. T. Gimingham	H. F. Barnes, W. Brown, T. Goodey, J. Henderson Smith, R. H. Stoughton
1940	W. B. Brierley	C. T. Gimingham	H. Wormald, H. Martin, F. R. Petherbridge, J. Henderson Smith, G. Fox Wilson
1941	W. B. Brierley	C. T. Gimingham	H. Wormald, H. Martin, F. R. Petherbridge, H. G. H. Kearns, W. C. Moore
1942	W. B. Brierley	C. T. Gimingham	H. Wormald, R. W. Marsh, F. R. Petherbridge, H. G. H. Kearns, W. C. Moore
1943	W. B. Brierley	C. T. Gimingham	F. C. Bawden, R. W. Marsh, F. R. Petherbridge, C. Potter, W. C. Moore
1944	W. B. Brierley	C. T. Gimingham	F. C. Bawden, R. W. Marsh, F. R. Petherbridge, I. Thomas, W. C. Moore
1945	W. B. Brierley	R. W. Marsh	F. C. Bawden, H. Martin, J. W. Evans, I. Thomas
1946	R. W. Marsh	I. Thomas	F. C. Bawden, H. Martin, J. W. Evans, D. J. Finney
1947	R. W. Marsh	I. Thomas	F. C. Bawden, H. Martin, J. W. Evans, D. J. Finney
1948	R. W. Marsh	I. Thomas	F. C. Bawden, H. Martin, J. W. Evans, D. J. Finney
1949	R. W. Marsh	I. Thomas	F. C. Bawden, H. Martin, D. J. Watson, D. J. Finney
1950	R. W. Marsh	I. Thomas	F. C. Bawden, H. Martin, D. J. Watson, D. J. Finney
1951	R. W. Marsh	I. Thomas	F. C. Bawden, J. T. Martin, D. J. Watson, F. J. Anscombe
1952	R. W. Marsh	I. Thomas	F. C. Bawden, J. T. Martin, D. J. Watson, F. J. Anscombe

ENVIRONMENTAL FACTORS CONTROLLING THE EVOCATION AND TERMINATION OF DIAPAUSE IN THE FRUIT TREE RED SPIDER MITE *METATETRANYCHUS ULMI* KOCH (ACARINA: TETRANYCHIDAE)

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(With 6 Text-figures)

Metatetranychus ulmi lays eggs of two types. The summer eggs, which are laid on the leaves of the host plant (e.g. apple), are of the non-diapause type and develop without interruption. The winter eggs, which are deposited predominantly on the bark, enter diapause at the blastoderm stage of development. 'Summer' and 'winter' females lay eggs of only one type if exposed to constant environmental conditions. Diapause is facultative. Over seventy successive summer generations have been reared under diapause-preventing conditions. Winter females appear in the first post-diapause generation in response to stimuli which induce diapause.

Three environmental agencies are capable of evoking diapause, namely photoperiod, temperature and nutrition. Mites feeding upon undamaged young or mature leaves obtain a plentiful food supply and the incidence of diapause is then determined solely by photoperiod and temperature. However, if the food supplies are restricted, winter females appear even when photoperiod and temperature are such as to prevent diapause. Such deficiencies, which are probably of a quantitative nature, are manifested when the diet consists of the cell contents either of senescing leaves or of 'bronzed' foliage previously damaged by the feeding punctures of large mite populations.

With daily photoperiods lasting from 6 to 13 hr., and at medium temperatures (c. 15° C.), only winter females develop. The incidence of diapause falls to zero at 15–16 hr., and only summer females are produced in continuous illumination. About 40 % of summer females appear in the absence of light. The mites respond to the absolute duration of the cycle of illumination and not to increasing or decreasing photoperiods.

Provided the illumination exceeds a threshold of 1–2 f.c., the response is independent of light intensity.

Radiation in the near ultra-violet, blue and blue-green regions of the spectrum is photoperiodically active. Maximum sensitivity occurs in the blue region. Wavelengths above 550 mμ, i.e. in the orange, red and infra-red regions, are totally inactive even if the energy level is high.

The mites are influenced directly by the photoperiod, not indirectly through the medium of the host plant.

High temperatures (e.g. 25° C.) tend to prevent diapause even if the photoperiod is short. Low temperatures (e.g. 10° C.) induce some diapause even with a long photoperiod. Temperature activity seems to be mainly confined to the dark phase.

Developing mites are indifferent to photoperiod, temperature and nutrition until the deutonymphal instar. This is also the period of greatest sensitivity, but egg-laying females, if exposed to antagonistic conditions, can still be caused to 'switch-

over' to the alternative egg type. Eggs intermediate in character may be laid during the period of reversal.

Winter eggs in diapause never hatch at 18 or 25° C. Diapause can be broken by chilling the eggs at 1, 5 or 9° C. for 150–200 days.

In *Tetranychus telarius* photoperiod, temperature and nutrition play a similar role in controlling the onset of diapause; and females in diapause can be caused to feed and oviposit if chilled. The tropical species *Metatetranychus bioculatus* is without diapause and photoperiod has no influence on development.

These experimental findings have been used in an interpretation of the life cycle and phenology of *M. ulmi*. In orchards where mite populations remain small throughout the season the first winter females appear at a time when food supplies are still plentiful. This is accounted for by the response to photoperiod.

INTRODUCTION

Two principal themes have been evident in recent studies of insect diapause. On the one hand, much insight has been gained both into the nature of this condition of arrested growth and into the humoral mechanisms which immediately govern its inception or termination. On the other, investigations concerned with the role of environment in controlling the onset of diapause, particularly in many species of Lepidoptera, have shown that one important agency, namely photoperiod, had been generally overlooked (Danilyevsky & Gayspitz, 1948; Dickson, 1949; Way & Hopkins, 1950). Photoperiod is also one of the factors which governs the onset of diapause in the fruit tree red spider mite *Metatetranychus ulmi* (Lees, 1950; Miller, 1950). Indeed, it would hardly be surprising if the connexion between diapause and daylength proved to be common among arthropods since this adaptation undoubtedly confers a stabilizing influence on the life cycle in environments where other significant climatic factors, such as temperature, are subject to much variation.

The diapause stage in *M. ulmi* is the winter egg. The summer egg, which differs in certain other particulars described below, develops without diapause. The characters of the eggs, however, are determined by the female mites. Although the latter are morphologically identical, experience has shown that each individual lays eggs of only one type provided the conditions during development, and subsequent to development, remain unchanged. It is therefore convenient to refer to 'winter' and 'summer' females. The incidence of diapause is then given by the proportion of winter females.

Three environmental factors which influence diapause are described in the following pages. The quite different conditions which cause the release of the winter egg from diapause have also been determined. The relevance of this information to phenological studies of the life cycle is discussed. Finally, the observations on *M. ulmi* have been extended to two closely related mites, namely *Tetranychus telarius* L. and *Metatetranychus bioculatus* Wood-Mason. The latter, a tropical species, was of interest in the present connexion since its geographical distribution includes areas where there is little or no seasonal change in the length of day.

MATERIAL AND METHODS

The mites were reared on small potted apple seedlings in cabinets constructed to contain about twenty such plants. Temperature could be controlled within $\pm 0.5^{\circ}\text{C}$. The illumination was provided by 2 ft. 20 or 40 W. fluorescent ('daylight') tubes operated by time switches. The intensity of the illumination falling on the upper leaves of the plants inside these cabinets varied from about 70 to 150 f.c., depending on the output and age of the tubes, distance of the plants from the light source, etc. Illumination of this order of intensity, although inadequate for healthy plant growth over long periods, was found to be sufficient to keep well-grown apple seedlings in good condition for the duration of the experiments (about 3 weeks). They were then discarded.

The usual experimental procedure was as follows. Five to ten summer females were placed on a clean seedling and allowed to oviposit for 3–4 days at a temperature of 25°C . and with a 16 hr. photoperiod. The mites were then removed. When the first protonymphs appeared 8 or 9 days afterwards the seedling was moved to the conditions under investigation. As soon as female mites developed they were transferred singly on the tip of a needle to other seedlings, and the type of egg laid by each individual was then recorded. Fifteen to twenty mites were tested in each experiment.

M. ulmi prepares to migrate by spinning a silken thread and hanging from the tip or underside of the leaf. With low population densities and in still air the mites show little tendency to leave the plants. There was therefore no need to enclose the seedlings in cages. Some difficulty was at first encountered with winter females which move about actively on the bark in search of an oviposition site. In order to deter them from walking off the stems of the rather small seedlings it was necessary to apply a ring of tanglefoot (by weight resin 8 pt., castor oil 5 pt., dimethyl phthalate 1 pt.) round the stem at soil level.

Recognition of summer and winter eggs

Although alike in general appearance (both are onion-shaped), the eggs can be identified by four characters. (i) The winter egg is slightly the larger. The diameter across the equator measures *c.* 0.15 mm. as compared with 0.14 in the summer egg. (ii) The bright red winter egg is more highly pigmented than the summer egg which shows considerable colour variation and may be almost colourless. (iii) The winter eggs are usually laid on the bark, whereas the summer eggs are almost invariably deposited on the leaves. These three characters usually suffice to distinguish the eggs, particularly if a sequence is examined, but in doubtful cases the eggs were incubated for 10 days at 25°C . (iv) Viable eggs in diapause neither hatch nor shrivel.

The choice of oviposition site (iii) involves striking differences in behaviour. The summer females are relatively inactive, often laying many eggs close together on the

leaf. The fact that the eggs are deposited along the veins and in other concavities suggests that the mites respond to contact stimuli in selecting the oviposition sites. The high humidity which doubtless prevails at the leaf surface seems to provide suitable conditions both for oviposition and for the subsequent development of the summer egg (Beament, 1951). Two to four eggs are laid daily at 15° C. No summer eggs are laid on the bark except in very overcrowded cultures.

The winter females feed quietly for some time on the foliage, but become highly active as the maturation of each egg is nearing completion. They then walk from the leaf and after many searching movements deposit the egg on the bark of the stem, often under the petiole or in crevices near dormant buds. Because of the constant alternation between leaf and bark, females are often seen feeding on a different leaf each day, in contrast with the summer females which may deposit all their eggs on one leaf. These movements are illustrated in Fig. 1 A, B.

This behaviour conveys the impression that the winter female is actively repelled from the leaf surface (perhaps by the relatively high humidity) when oviposition is imminent. However, the restraint is not absolute, for in a series of 328 eggs laid by winter females twenty-three (7%) were deposited on the leaves. This 'error' of oviposition was particularly marked in a few individuals which laid over half their eggs on the leaves; the majority deposited every egg on the bark. The oviposition rate at 15° C. of about one winter egg daily is much lower than in the summer females.

The plants

The moistened apple seed was exposed to a temperature of 10° C. to break dormancy. Under these conditions germination takes place within 2-4 months. If required, the germinated seed could afterwards be stored at 1° C. for a month or so longer. By planting up germinated seed at intervals uniform batches of seedlings could be obtained at any time of year. During the winter, when the plants were grown in a heated glasshouse, a 140 W. sodium vapour lamp was employed to extend the daily photoperiod to 16 hr. Supplementary illumination of high intensity was found to be essential for promoting adequate vegetative growth in winter. The plants were sprayed regularly with an organic copper fungicide to suppress mildew (Massee, 1947).

The seedlings used for rearing mites varied in age from 2 to 5 months. Both young, growing leaves and mature, deep green leaves which have ceased to expand are to be found on plants of this age. In experiments dealing with factors other than nutrition care was always taken to ensure that no senescent (yellowing) foliage were present, as this leaf condition exerts a marked influence on diapause.

THE NATURE OF DIAPAUSE IN *METATETRANYCHUS ULMI*

In certain insects, for example the Agrotid moth *Mamestra brassicae* (Way, Smith & Hopkins, 1951) the tendency to enter diapause seems to increase with each successive generation, independently of the environmental conditions. In order to determine

whether diapause in *M. ulmi* is influenced by the number of successive summer generations through which the stock has passed mites were maintained continuously under conditions of high temperature (25° C.) and long day-length (16 hr.). This line was started with a single fertilized summer female of the first

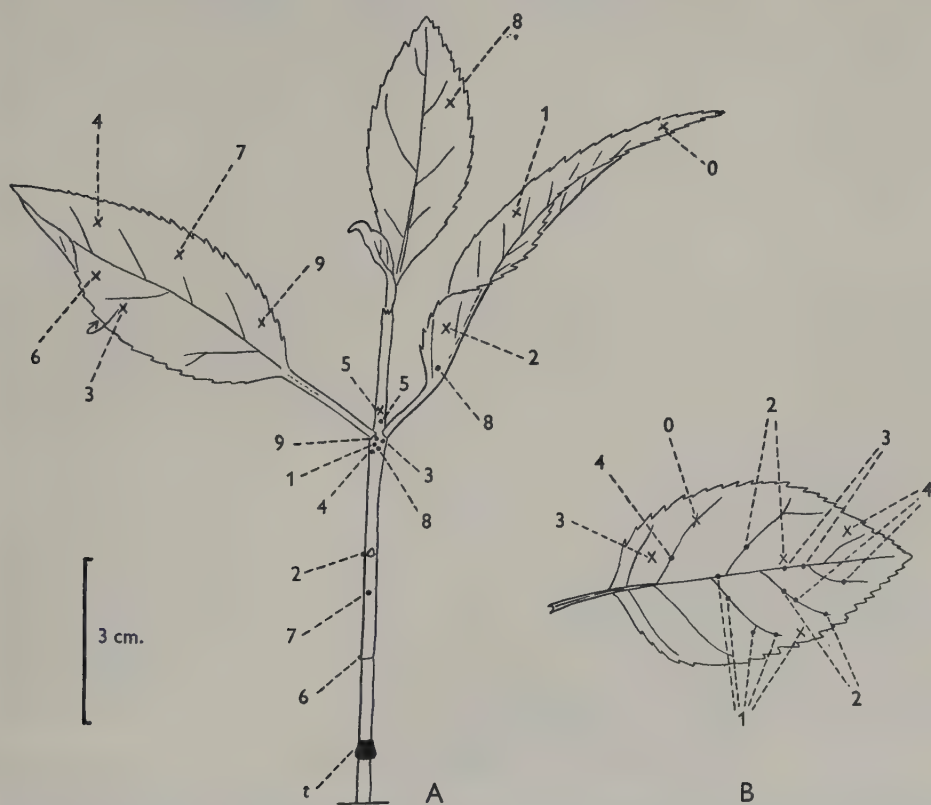


Fig. 1. The egg-laying behaviour of *M. ulmi*. A, the movements of a winter female when laying a sequence of ten winter eggs on a small apple seedling. B, a summer female laying eleven summer eggs on a single leaf. Positions of eggs and of the mites themselves at the moment of the daily observation are indicated by dots and crosses respectively. The chronological order is shown by the numbers which refer to the day of the observation. *t*, tanglefoot.

post-diapause generation. The winter egg from which this mite hatched had been part of a large batch collected in Essex. When the mites had passed through fifteen to nineteen successive summer generations their response to specific diapause-inducing or diapause-preventing conditions was compared with that of mites which had just hatched from winter eggs.

The results (Table 1) show that there is no difference in behaviour. At 15° C. the appropriate stimuli, such as short photoperiods of 8 or 12 hr. are as effective in

TABLE 1. *The reactions of mites (Metatetranychus ulmi) which have passed through only one or through many consecutive summer generations to diapause-inducing or diapause-preventing conditions*

Temp. (° C.)	Photoperiod (hr./24 hr.)	No. of generation	% winter females
15	8	1	100
		16	100
	12	1	96
		15	100
	16	1	0
		15	0
25	8	1	20
		18	29
	12	1	15
		19	27

inducing diapause in 1st generation mites as in those of the 15th and 16th generation. Similarly, a long photoperiod is as effective in preventing diapause in the 15th as in the 1st generation. If the stimuli are conflicting, as when a high temperature is accompanied by a short photoperiod, the progeny include both summer and winter females. Nevertheless, the proportion in which they occur is not significantly affected by the generation number.

The induction of diapause in 1st generation mites shows that the offspring of winter females can become winter females without the interpolation of summer generations. Conversely, after 3 years under diapause-preventing conditions, mites from the stock culture had passed through seventy-one successive summer generations without the appearance of winter females. The probability that diapause will occur in any given generation is evidently neither enhanced nor diminished by the diapause history of the previous generations. Diapause is therefore of the facultative type and is governed only by extrinsic agencies.

In practice, mites of the 13th to the 65th generations in the stock culture formed the principal source of material, but 1st generation larvae were also used on occasion.

FACTORS CONTROLLING THE ONSET OF DIAPAUSE IN *METATETRANYCHUS ULMI*

The photoperiod

The influence of day-length is most clearly manifested at medium temperatures. The incidence of diapause at 15° C. and with different photoperiods of constant duration is shown in Table 2 and Fig. 2. With photoperiods lasting from 6 to 13 hr.

TABLE 2. *The induction of diapause by photoperiod in Metatetranychus ulmi (Temperature, 15° C.)*

Photoperiod (hr./24 hr.)	0	4	6	8	12	13	14	15	16	24
% winter females	60	85	100	100	97	94	54	4	0	0

(and with complementary dark periods of 18–11 hr.) diapause is virtually universal. As the photoperiod is extended from 13 to 15 hr., and the complementary dark phase reduced from 11 to 9 hr., the incidence of diapause falls sharply to 4%. A photoperiod of about 14 hr. is therefore critical. No winter females develop with a 16 hr. photoperiod or in conditions of uninterrupted illumination.

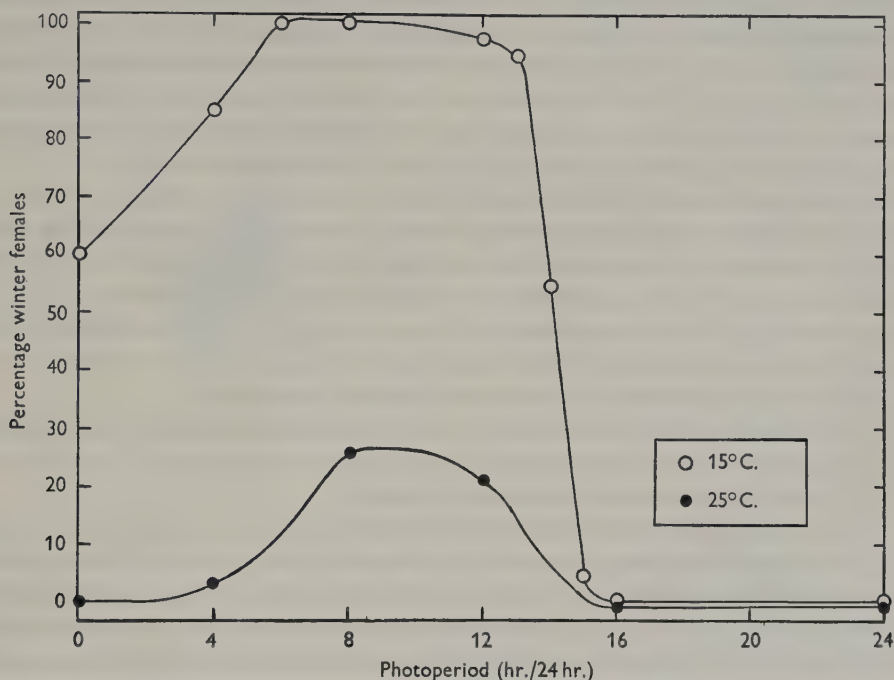


Fig. 2. Influence of photoperiod on diapause in *M. ulmi* at constant temperatures of 15 and 25° C.

Summer females again appear with photoperiods shorter than 4 hr., this trend being most marked in the absence of light. By selecting strong plants with mature leaves it was possible to rear the mites in complete darkness except for one period of about 10 min. when the females were transferred to separate seedlings. In three replicated experiments in which a total of fifty mites were tested, the proportion of winter females ranged from 80 to 47% (mean 60%). It is possible that this variability is due to the differing nutritive value of the leaves of plants kept continuously in darkness. A serious deficiency in food supplies does in fact lead to a marked increase in the incidence of diapause (see p. 465). Danilyevsky (1948) has recorded similar variations in *Acronycta rumicis* when this Agrotid moth was reared in virtually complete darkness, even though the food plant was changed daily.

The action of photoperiod in *Metatetranychus ulmi* is very similar to its action in several Lepidoptera, although in the latter the maximum differential effect of

typical 'short' and 'long' days is usually exerted at a somewhat higher temperature. Curves resembling that in *M. ulmi* at 15° C. have been obtained in the silkworm at 15° C. (Kogure, 1933), in *Acronycta rumicis* at 27–28° C. (Danilyevsky, 1948), in the Agrotid *Diataraxia oleracea* at 24° C. (Way & Hopkins, 1950), and in *Grapholitha molesta* at 24° C. (Dickson, 1949). However, the following differences may be noted (i) The direction of the response in the silkworm is reversed; the curve is therefore inverted (see p. 485). (ii) Slight but significant differences occur in the 'critical photoperiod', i.e. the natural day-length at which the incidence of diapause changes most rapidly (p. 485). (iii) The reaction to the absence of light differs. *Bombyx mori* responds no differently than to a short day. In *Diataraxia* about 80% of the insects enter diapause; in *Acronycta* about 20%; and in *Grapholitha* only 2%.

Increasing and decreasing photoperiods. Since the natural day-length is subject to progression it was thought advisable to test the influence of photoperiods that were regularly shortened or lengthened during the developmental period of the mites. At a latitude of 52° C. the maximum daily increment and decrement is 4 min. The longest day-length (sunrise to sunset) is 16 hr. 45 min. and the shortest, 7 hr. 45 min. In the following experiments, which were carried out at 15° C., the mites were in the larval stage at the time of exposure to the first photoperiod.

(a) The initial photoperiod of 16 hr. 45 min. was shortened by 5 min. daily. Egg-laying females were available for testing when the photoperiod had fallen to 15 hr. 35 min. All proved to be summer females.

(b) The initial photoperiod of 8 hr. was lengthened by 7 min. daily. The female mites were tested when the photoperiod had reached 9 hr. 44 min. All were winter females. Developing mites were also exposed to a 12 hr. photoperiod falling by 10 min. stages to 9 hr. 20 min. This experiment also yielded only winter females.

In nature the first summer generation develops in late spring when the hours of daylight are still increasing rapidly, while winter females appear as they are diminishing in early autumn. Nevertheless, it is clear that this progression is without influence on diapause. A short but rapidly lengthening photoperiod does not promote development nor does a long but rapidly shortening photoperiod favour the diapause condition. *Metatetranychus ulmi* therefore resembles *Grapholitha molesta* (Dickson, 1949) in responding only to the absolute length of the photoperiod.

Intensity of illumination. In determining the sensitivity of the photoperiodic response it would have been possible to make use of the fact that the incidence of diapause in continuous darkness differs from its incidence under short- or long-day conditions. However, in view of the variability encountered with this treatment, and also because of the detrimental effect on the plants of single photoperiods of low intensity, the method illustrated in Fig. 3 was adopted.

Two similar light sources, both 'natural' fluorescent tubes, were employed. The first illuminated the plants at a standard intensity of 90 f.c. The illumination of the second source was reduced as required by surrounding the tube with black paper rings of variable number and width. This preserved a very even field of illumination.

The seedlings bearing developing mites were exposed to a daily regime of 8 hr. light of standard intensity, 8 hr. light of reduced intensity and 8 hr. of darkness. The circuit controlling this cycle was so arranged that the second tube was switched on only a few seconds after the first was switched off. The intensity of illumination was measured with a selenium cell photometer calibrated against a standard tungsten filament lamp. The intensities recorded in Fig. 3 are those at the upper surface of the leaves (which were usually reduced to a single tier); little more than one-tenth of this illumination was reaching the undersurface where the mites frequently feed.

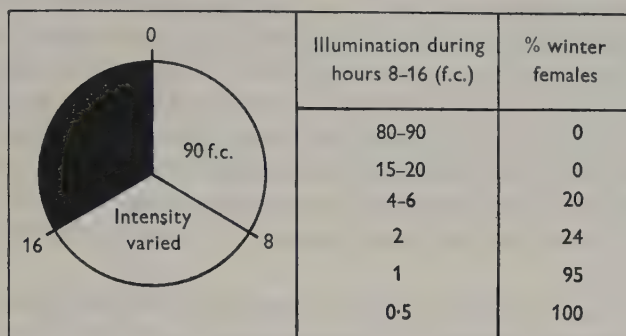


Fig. 3. Intensity of illumination and the photoperiodic response. The daily regime of light and darkness is indicated on the left.

The threshold of perception is in the region of 1-2 f.c. Fig. 3 shows that when the illumination from the second (variable) source is greater than this figure the mites respond to a 16 hr. photoperiod and develop as summer females; if the light intensity is below the threshold the mites respond only to the 8 hr. photoperiod from the standard source and therefore develop as winter females.

Provided the illumination exceeds the threshold, the incidence of diapause is affected only by the duration of the photoperiod and is independent of intensity and total light energy. In one experiment mites were reared either with an 8 hr. photoperiod and a light intensity of 90 f.c. or with a 16 hr. photoperiod and a light intensity of 45 f.c. Although the total light energy was constant only winter females were produced under the first conditions, only summer females under the second.

A similar relationship between intensity of illumination and the photoperiodic response has been demonstrated in several Lepidoptera. The threshold of sensitivity in the silkworm is about 0.01 f.c. in the egg and 0.08 f.c. in the young larva (Kogure, 1933); in *Acronycta rumicis* it is below 0.5 f.c. (Danilevsky, 1948); in *Diataraxia oleracea* below 1 f.c. and in *Grapholitha* between 1 and 3 f.c. (Dickson, 1949).

Spectral sensitivity. Since preliminary trials showed that *Metatetranychus ulmi* was far from uniformly sensitive to different wave-lengths in the visible spectrum,

an attempt was made to estimate, in relative terms, the minimal energy required for eliciting the photoperiodic response.

Method. The procedure for testing radiation for photoperiodic activity was the same as in the previous experiment. The daily light regime consisted of 8 gr. standard illumination of 90 f.c. from a 'natural' fluorescent tube, 8 hr. irradiation from the second (experimental) source and 8 hr. darkness. The temperature was 15° C.

For investigating the region of the spectrum extending from blue to the infra-red a 500 W. general lighting service lamp was used as the second source. To reduce heating effects this lamp was enclosed in a light-tight metal housing through which cool air could be rapidly circulated by means of a 2 in. centrifugal fan. For testing the sensitivity to ultra-violet radiation an 125 W. high-pressure mercury vapour lamp was substituted for the tungsten filament lamp. Radiation of the required wave-length was obtained by passing the beam through 4 in. square glass filters which were held in a filter carrier mounted in the base of the lamp housing.

Light absorption by this series of filters was measured spectrophotometrically. The salient characteristics (limits, peak) are given in Table 3; the 5% level is here

TABLE 3. *The relative spectral sensitivity of the photoperiodic reaction in Metatetranychus ulmi*

(Note that winter females are expected if the radiation stimulus is not perceived. For further explanation, see text.)

Light source	Filters	Wave-lengths transmitted by colour filter		Relative energy of wave-lengths shorter than 700 m μ (arbitrary units)	Illumination (f.c.)	% winter females
		Limits (m μ)	Peak (m μ)			
Tungsten filament lamp	Signal red (Chance OR2) + ON2O	Above 580	700 and above	830	150	100
	OR2 alone	Above 580	700 and above	1246	155	98
	Light orange (Chance OY2) + ON2O	Above 530	650 and above	1200	175	100
	Signal green (Chance OGr1) + ON2O	480-600	540	143	100	0
				9	6	26
				4	2.5	87
	Blue (Chance OB1O) + ON2O	350-500	425	89	4	0
				15	0.7	0
				6	0.2	6
				1	0.03	27
Mercury vapour lamp	Ultra-violet (Wratten 18A) + ON2O	300-390	365	516	0	0
				52	0	12
				15	0	100

regarded as marking the effective limits of transmission. As all the filters transmitted up to 90% of the radiation in the infra-red ($1-4\mu$), it was necessary to use them in conjunction with a clear heat-absorbing filter (Chance ON20). While transmitting 70-90% of the visible radiation the latter allowed less than 5% of the infra-red radiation to pass through. Ventilation of the lamp housing and the use of the heat-absorbing filter permitted a high energy beam to be used without disturbing the temperature control in the plant cabinet.

The estimation of the relative energy reaching the upper surface of the leaves was made as follows. The 210 V. tungsten filament lamp, which was run at a mains voltage of 200 V., was assumed to burn at approximately 2865°K . The known spectral energy distribution of similar lamps at this colour temperature could therefore be taken as the basis of calculation. Knowing the filter characteristics, the relative energy falling on the plants could then be obtained graphically. Since the mercury vapour lamp has an entirely different energy distribution, the selenium photocell of known spectral sensitivity was used in comparing the energy emitted by the two sources. The relative energy of the radiation of wave-lengths shorter than $700\text{ m}\mu$ (an arbitrary point corresponding to the upper limit of the human visibility curve) is entered in Table 3.

The intensity of the illumination in foot-candles was also measured with photocell and compensating filter. Although these determinations are included for general reference in Table 3 this measurement has no particular significance in relation to the spectral sensitivity of the mite.

A series of experiments employing each filter in turn was first run with the lamps at the minimum working distance from the plants. If a positive response was recorded the intensity of the radiation was progressively reduced until the threshold of sensitivity was reached. The reduction was usually brought about by increasing the distance between the lamp and the plants but occasionally a screen of perforated zinc was employed.

Results. The mites proved to be completely insensitive to high intensity radiation in the red and infra-red regions of the spectrum. With the tungsten filament lamp at the shortest possible working distance (about 6 in. from the plants), the red filter in combination with the infra-red filter ON20 transmitted approximately 830 units of energy between 580 and $700\text{ m}\mu$ and 2110 units between 700 and $2500\text{ m}\mu$ (the latter approaching the upper limit of emission of tungsten at the colour temperature in question.)

Without filter ON20 the relative energy of the radiation in the red and infra-red regions of the spectrum was far higher, namely 1250 and 23,880 units respectively. Indeed, the radiant energy was so considerable that the cooling system proved inadequate, the temperature in the plant cabinet rising from 15 to 20°C . during the 8 hr. period of irradiation. However, this was insufficient to influence diapause which remained virtually universal under both conditions.

Since high energy levels in the infra-red are without effect on diapause, the con-

siderable content of infra-red radiation transmitted by the light orange, green, blue and ultra-violet filters can be ignored. For this reason, only wave-lengths shorter than $700\text{ m}\mu$ have been considered.

Table 3 shows that there was no response to photoperiod with orange light of the maximum possible intensity. But diapause was influenced by high intensity radiation in the green, blue and near ultra-violet regions of the spectrum. In each case the developing mites responded to a 16 hr. photoperiod and became summer females.

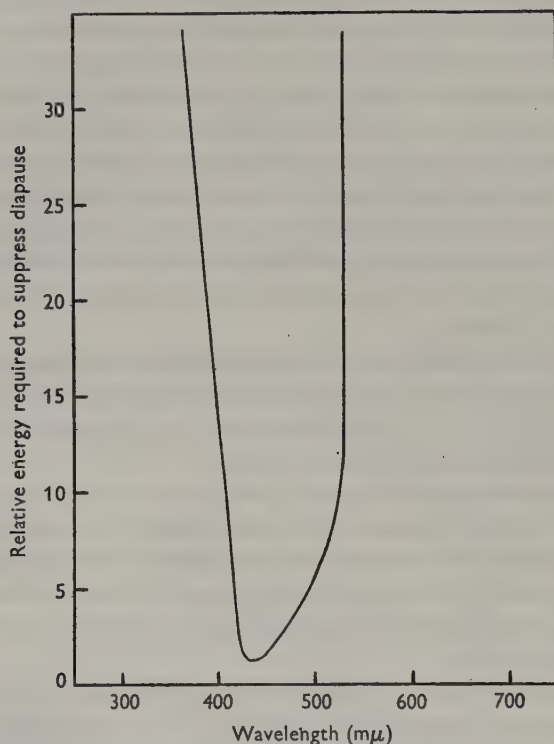


Fig. 4. Action spectrum curve showing the approximate relative energy required at different wave-lengths to suppress diapause in *M. ulmi*.

The relative sensitivity to energy in these effective regions was obtained by reducing the intensity until the mites responded only to the 8 hr. photoperiod from the standard source. The threshold is reached when half the mites become winter and half summer females. The relative energy required to produce this response was found to be approximately in the ratio near ultra-violet 34:blue 1:green 7. This relationship has also been expressed as an activity curve (Fig. 4) although, in view of the non-selective nature of the filters, this is an approximation only.

Whilst the greatest sensitivity is shown to wave-lengths in the blue region, there is little doubt that the response to near ultra-violet and green is also genuine. Thus

with the ultra-violet source and filter 95% of the total energy was transmitted at the 365 m μ mercury line which lay outside the limits of transmission of the blue filter. The degree of overlapping of the blue and green filters was also very slight. Although the light orange filter had a transmission factor of 30% in the green at 555 m μ these wave-lengths were without influence on diapause. The effective radiation transmitted by the green filter is therefore probably to be found in the blue-green (500–525 m μ) region.

These results are in general agreement with previous observations on Lepidoptera. Kogure (1933) found that violet light (350–510 m μ) exerted the greatest photoperiodic activity in the silkworm; orange-yellow (above 550 m μ) was only slightly active and red completely inactive. Dickson (1939), while recording no response either with ultra-violet radiation or red light, showed that *Grapholitha molesta* was most sensitive to the blue-green region of the spectrum.

Parker, Hendricks, Borthwick & Jenner (1952) have recently suggested that the spectral sensitivity of the photoperiodic reactions in plants (control of flowering, promotion of leaf elongation, etc.) and animals (diapause, reproductive cycle in birds and mammals) may show fundamental similarities. In several plant species the effect is greatest in the red wave-lengths (620–660 m μ) and declines to a minimum in the blue-green region (460 m μ). These authors point out that activity curves of this type show some correspondence with the absorption spectra of compounds like phycocyanin, which contain tetrapyrrol groupings. They also go further and suggest, on the basis of the rather scanty evidence available in the vertebrates, that the action spectrum in animals may have similar characteristics; and this they correlate with the recorded presence of coproporphyrins in the central nervous system of birds and mammals. However, so far as the arthropods are concerned, it is evident from the entirely different action spectrum that some other photodynamic agent will have to be sought.

The role of the host plant in the control of diapause by photoperiod

As substances under the control of photoperiod are known to occur in plants, it is conceivable that diapause in the mite is influenced by the light treatment which the host plant receives. This was tested with two groups of apple seedlings. Each group was exposed daily to 8 hr. of light and 16 hr. of darkness at 15° C., but the lighted phases were arranged so as to follow on consecutively. The mites (including moulting stages which were carefully detached from the leaves) were transferred twice daily from one group to another in the manner indicated in Fig. 5 A. The net result was that while both groups of plants were exposed to an 8 hr. photoperiod, the mites experienced a 16 hr. photoperiod. Thirty-eight female mites were reared and all proved to be summer females. Clearly, the host plant is not concerned in the mediation of the photoperiodic response in *Metatetranychus ulmi*.

Although the mites are directly influenced by light there remain several possibilities concerning its mode of operation. The photoperiod may be perceived

through some photosensitive 'receptor'; or alternatively, light may affect diapause more indirectly through the medium of a second factor, for example, feeding activity. This has to be considered in *M. ulmi* as nutrition plays an important role in the induction of diapause.

It is not known whether *M. ulmi* feeds more actively in light or in darkness. The following experiments were intended to allow for either eventuality. In the first

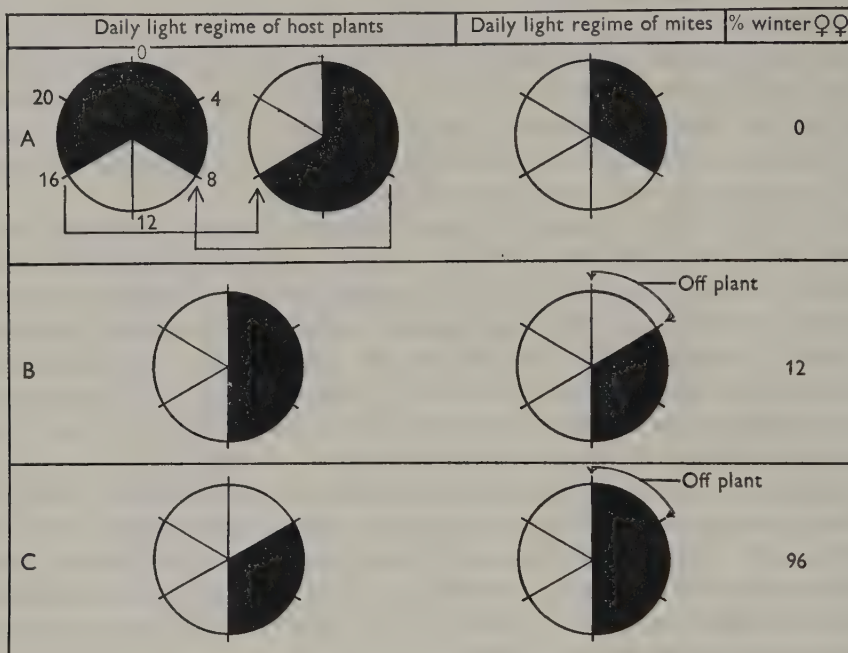


Fig. 5. Methods of handling mites (*M. ulmi*) in experiments designed to show that the photoperiodic response is not dependent upon nutritional changes in the host plant.

experiment (Fig. 5 B) the host plants received a daily regime of 12 hr. light and 12 hr. darkness. The developing mites were allowed to feed for 12 hr. daily during the illuminated period and were next transferred from the plant to an illuminated glass tube for a further 4 hr. period. They were then returned to the plant for 8 hr. of darkness. Thus they received a 16 hr. photoperiod, but their opportunities for feeding in the light were restricted to 12 hr.

Out of twenty-five mites reared twenty-two were summer females. In general, therefore the mites are responding directly to the long photoperiod. If the time available for feeding in the light were a significant factor diapause should have been universal—as would have been the case if the mites had remained continuously on the host plants. The appearance of a small proportion of winter females in this experiment may have been the result of the regular daily period of starvation (see pp. 464 et seq.).

In order to test the less probable assumption that diapause is influenced by the feeding activity during the dark phase the experiment indicated in Fig. 5C was carried out. The plants were illuminated for 16 hr. while the mites were subjected to 12 hr. of illumination on the plants, 4 hr. starvation in a darkened tube and 8 hr. darkness on the plants. Although the mites thus experienced a 12 hr. dark period daily, opportunities for feeding in darkness were restricted to 8 hr.

Among twenty-eight females reared twenty-seven were winter females. Clearly, diapause is influenced directly by the long dark phase and not by the feeding activity during the hours of darkness.

The result of experiment C also supports the conclusion that diapause is not controlled through the medium of the plant since the plants received a long-day and the mites a short-day regime.

Temperature

This factor also strongly influences the onset of diapause. The results obtained when mites were reared at constant temperatures of 10, 15, 20 and 25° C., and with different photoperiods, are set out in Table 4. The 15 and 25° C. curves have also been plotted in Fig. 2.

TABLE 4. *The influence of temperature and photoperiod on the incidence of diapause (% winter females) in Metatetranychus ulmi*

Temp. (° C.)	Photoperiod (hr./24 hr.)					
	0	4	8	12	16	24
10	91	90	100	100	45	0
15	60	85	100	97	0	0
20	36	54	72	70	0	0
25	0	3	27	21	0	0

The two higher temperatures favour the non-diapause condition. This is, of course, most apparent when the cycle of illumination is diapause-inducing. Thus with photoperiods of 8 and 12 hr. the incidence of winter females was reduced to 27 and 21 % respectively.

Low temperatures exert the reverse effect, tending to increase diapause at nearly all photoperiods. At 10° C. (a temperature only slightly above the threshold of development) and with a 'long-day' of 16 hr., winter females made up 45 % of the total even though this photoperiod suppresses all diapause at medium temperatures. The tendency of low temperature to induce diapause can nevertheless be overcome by still longer photoperiods for only summer females developed at 10° C. when the illumination was continuous.

In the silkworm (Kogure, 1933) and in *Diataraxia* (Way & Hopkins, 1950) high temperatures and long photoperiods also act in concert as do low temperatures and short photoperiods. In *Grapholitha*, however, both high and low temperatures seem to favour development without diapause (Dickson, 1949).

The action of temperature in relation to the cycle of light and darkness. Temperature activity may either bear some relation to the cycle of illumination or, as Way & Hopkins (1950) have suggested, may be wholly independent of it. Some evidence on this point has been provided by rearing mites in a diurnally fluctuating temperature. The light regime consisted of equal 12-hourly periods of light and darkness. The 12-hourly periods of constant high temperature (25° C.) or constant medium temperature (15° C.) were timed to coincide with either the dark or the light phase.

TABLE 5. *The action of temperature in relation to the light and dark phases of the cycle of illumination*

(The influence of the diapause-preventing temperature (25° C.), when coincident with either phase (above), is compared with that of the same temperature when continuously maintained (below).)

Cycle of illumination		% winter females
12 hr. light	12 hr. darkness	
Temp. (° C.)		
25	15	96
15	25	53
15	15	97
25	25	21

The results show that the relationship is not a simple one (Table 5). A high temperature during the light phase is entirely without influence, almost every mite still developing as a winter female. When the high temperature is given during the dark phase the incidence of diapause is reduced to 53%. But it is unlikely that temperature activity is confined to the dark phase for there is a further fall to 21% when the entire period of development is spent at the higher temperature. This result suggests that in *Metatetranychus ulmi* there is some kind of interaction between temperature and the cycle of illumination.

Nutrition

Observations in commercial orchards in Holland (Kuenen, 1946) and England (Blair, 1951) have established that when heavy infestations of red spider mite have caused severe foliage injury ('bronzing') winter eggs appear on the trees at an unusually early date in the summer. Most workers have concluded that nutrition plays an important role in the determination of egg type; indeed, this has sometimes been thought to be the only factor involved.

If the diapause behaviour is influenced in this manner by the available food supplies it would not be surprising if it were also affected by the cycle of growth and senescence of the foliage on the host plants. In the experiments described below mites were reared on different types of apple foliage under known conditions of temperature and photoperiod.

Physiological leaf age. For present purposes only three stages have been distinguished: (i) *Young leaves* are small but rapidly growing. They are light green in colour and soft in texture. (ii) *Mature leaves* are fully expanded, deep green in colour and are no longer flaccid. (iii) *Senescent leaves* are green with patches of yellow. In the final stages of senescence the leaf becomes completely yellowed.

One-year-old or current year apple shoots bearing leaves of the required growth stage were cut from trees (varieties Worcester and Annie Elizabeth) growing in a Cambridge orchard. The dates of collection in spring and early autumn are given in Table 6. During September it was possible to collect both mature and senescing

TABLE 6. *The influence of the food source (apple leaves of different physiological age) on diapause in Metatetranychus ulmi*

Leaf age	Date of collection	Temp. (° C.)	Photoperiod (hr./24 hr.)	% winter females
Young	20 May	15	16	0
		15	8	95
		25	16	0
Mature	30 Sept.	15	16	0
		15	8	94
		25	16	0
Senescing	30 Sept.	15	16	68
		15	8	100
		25	16	12

foliage on the same day. The cut shoots were then inserted in moist sand in the laboratory and infested with mites in the manner previously described. Egg-laying females were tested individually on separate shoots bearing leaves of the same growth stage.

The influence of physiological leaf age is shown in Table 6. When the food consists of young or mature leaves the incidence of diapause is exactly as would be expected from a consideration of the temperature and photoperiod only. Clearly, the act of feeding on leaves of this type does not favour either the assumption of the diapause or the non-diapause condition. This is shown most strikingly when mites are reared on young spring foliage at a medium temperature (15° C.) and with a short 8 hr. photoperiod. In nature foliage of this type would provide the food for mites of the first summer generation which develop into summer females exclusively. Yet under the influence of a short photoperiod 95% of winter females appeared.

M. ulmi cannot survive on completely yellowed leaves, but when senescence is incomplete and some green areas remain, the nutritive content of the leaf is still sufficient to permit development. But there is now a marked influence on diapause, this condition tending to induce diapause strongly. 68% of winter females were recorded with a long photoperiod and a medium temperature, and even when both photoperiod and temperature tend to prevent diapause, a variable proportion of winter females still appears (Table 6). Evidently the influence of temperature and

photoperiod can be partially or completely overcome by a quantitative or qualitative deficiency in the food supplies.

The mechanism of feeding in *M. ulmi* is relevant in this context. The accounts of this process by Kuenen (1946), Blair (1951) and Blair & Groves (1952), have established that the inserted stylets pierce the leaf epidermis (usually on the under-surface near a vein) and after passing through the spongy mesophyll, penetrate individual cells in the palisade layer. The chloroplasts and other contents are then sucked out.

Dependence on the cell contents as the source of food may account for the fact that the young foliage used in the present experiments proved to be much less favourable for growth than mature leaves. The mortality was high (the dense pubescence of young leaves from fully grown orchard trees may have been partly responsible) and growth was considerably retarded. Females required 22–35 days at 15° C. to develop from the larval stage as compared with 15–16 days when the food consisted of mature foliage. Evidently the nutritive value of the cell contents in young leaves is relatively poor but not sufficiently so to influence diapause. The deficiency is probably much greater in partially senescent leaves, possessing few functional chloroplasts.

It is noteworthy that the differing food value of foliage is also reflected in the feeding behaviour of the mites. The usual feeding preference is for mature leaves. Young and senescing leaves are avoided. Sucking insects which obtain their food directly from the sieve tubes in the vascular bundle behave very differently. In aphids, for example, young and senescing leaves are more favourable than mature leaves for colonization and reproduction (Kennedy, Ibbotson & Booth, 1950).

Bronzing. The premature deposition of winter eggs in commercial orchards carrying heavy mite infestations has shown that extensive leaf injury must also induce diapause. This has been confirmed in the laboratory under controlled conditions of temperature and photoperiod.

The bronzed appearance of the foliage is caused by the removal of the chloroplasts from individual palisade cells. The remaining cell contents then coagulate forming an amber-coloured mass at one end of the cell (Blair, 1951). Although the preferred feeding sites lie along the veins of the leaf, dense populations of feeding mites become more scattered. The damaged palisade cells are therefore distributed sufficiently evenly over the leaf surface to give a relatively uniform bronzed appearance. This circumstance permits the degree of bronzing to be judged roughly by eye. The damaged leaves are extracted in alcohol, cleared in xylol and mounted in balsam. The intensity of damage has been judged by comparison with a graded series of damaged leaves from an Essex orchard prepared by Miss C. A. Blair (Blair, 1951, Fig. 1). This series extends from undamaged (stage 1) to badly bronzed (stage 6) leaves. In the field the first winter eggs were laid on trees with foliage showing grade 5 damage.

These conditions were reproduced in the laboratory by infesting apple seedlings

with large numbers of mites in all stages of development and by permitting the population to build up without restriction. The plants were then exposed to conditions of photoperiod and temperature that would normally prevent diapause. When the first winter eggs appeared, samples from one typical leaf on each plant were prepared and graded. On ten plants maintained at 15° C. and with a 16 hr. photoperiod the intensity of damage was rated as follows:

4, 4, 4, 5, 5, 5, 6, 6, 6, 6,—mean = 5.1.

With the same photoperiod but at a temperature of 25° C. winter eggs were not usually deposited until bronzing was slightly more extensive:

4, 5, 5, 6, 6, 6, 6, 6, 6, 6—mean = 5.6.

Although damage of a certain degree of severity can evidently overcome the influence of both high temperature and long photoperiod, the results suggest that with somewhat less intense bronzing the effect of temperature on diapause will still be perceptible. The interaction with temperature was followed further by rearing small batches of mites on the same host plant but at the two different temperatures. A seedling showing stage 5 bronzing which under long day conditions was producing 28% winter females (twenty-five mites tested) at 25° C. was cleared of mites and reinfested with protonymphs. When these were reared at 15° C. 74% proved to be winter females (forty-two mites tested).

Although the degree of bronzing was not visibly intensified by the feeding activities of this small number of mites, a reciprocal experiment was also carried out. A bronzed seedling of stage 4 which was producing 38% of winter females at 15° C., after clearing and reinfestation, subsequently yielded only 19% of winter females at 25° C. It seems that if the food intake falls below a certain threshold, diapause is induced regardless of photoperiod and temperature. But those mites which obtain food in excess of this amount may still be influenced by these factors.

It will have been noted that the act of feeding on bronzed or senescent foliage affects diapause in a like manner. Yet there is no reason to suppose that the chemical changes which characterize these leaf conditions have anything in common. Although mite damage eventually causes premature leaf fall in the field, severely bronzed leaves are often borne for long periods on seedlings without senescence or abscission occurring. In both cases the effect on the mite may be one of partial starvation.

The influence of starvation. An attempt was therefore made to induce diapause by removing the mites from the host plants. However, when deutonymphs and teneral females were deprived of food during a 16 hr. period, all the mites subsequently became summer females when the conditions of photoperiod otherwise tended to prevent diapause. One period of starvation, even if this approaches the limits of tolerance, is therefore insufficient to influence diapause. Probably the depleted reserves are rapidly rebuilt when the mite is returned to the plant and has access to unlimited food supplies. On the other hand, less extreme but more

regular periods of starvation appear to be effective. When mites were deprived of food for 4 hr. daily throughout development 12% became winter females although they had experienced a 16 hr. photoperiod (see p. 462).

Interaction of the factors influencing the onset of diapause

It is apparent that there are substantial differences in the mode of interaction of these agencies. Photoperiod and temperatures seem always to exert a positive influence on diapause, favouring, in some measure, either one or the other of the alternative potentialities of development. In combination these factors behave in an additive manner. Thus the most potent diapause-preventing stimulus is a long photoperiod combined with a high temperature. A long photoperiod combined with a medium temperature, or a short photoperiod combined with a high temperature, are somewhat less effective in this respect. And diapause is induced most strongly by a short photoperiod when combined with a low temperature.

Nutrition, on the other hand, appears to operate as a limiting factor. When the food supplies are plentiful nutrition exerts no influence on diapause. When they are inadequate—and adequacy of senescent or bronzed foliage may be determined by the density of the palisade cells containing functional chloroplasts—the path of development is deflected towards the diapause condition, even though temperature and photoperiod may tend to prevent diapause strongly.

THE SENSITIVE STAGES OF DEVELOPMENT

The time of operation of the factors governing the onset of diapause was determined by transferring mites in different stages of development from diapause-inducing to diapause-preventing conditions and vice versa. Ovipositing females which had been influenced by the conditions after transfer could be discarded immediately. But when it became apparent that the type of egg laid, even by mature females, was not irrevocably fixed, it became necessary to follow the complete course of oviposition if the eggs showed the influence of the first and not that of the second and current treatment.

Records for ten individuals of each stage were compiled. As the mites were sometimes lost, this is necessarily a selection from a somewhat larger number of records. Since, also, the spent females were rarely found, records were only chosen for inclusion if oviposition could be presumed to be complete or nearly so. In Tables 7 and 8-11 the number of eggs laid and the time interval before the deposition of the last egg provide some indication of the completeness of the observation. According to Blair & Groves (1952) summer and winter females both lay an average of twelve eggs.

As the eggs and larvae were found to be entirely insensitive to photoperiod and temperature the only stages to be considered are the protonymph, the deutonymph and the female. Both teneral and older, ovipositing females were examined.

Photoperiod

The effect of transferring mites from an 8 hr. (diapause-inducing) to a 16 hr. (diapause-preventing) photoperiod and vice versa at 15° C. is shown in Table 7.

Protonymphs clearly remain uninfluenced by the initial light regime, their development being in accordance with the conditions which they experience subsequently. The same is usually true of active deutonymphs. In every female mite transferred

TABLE 7. *The sensitivity of different developmental stages of Metatetranychus ulmi to photoperiod*

(Ten mites in each category were transferred from a diapause-inducing 8 hr. photoperiod to a diapause-preventing 16 hr. photoperiod (A), or vice versa (B). Temperature, 15° C. throughout.)

Stage transferred	No. of mites laying				Mites responding to the second and antagonistic photoperiod			Mites not responding to antagonistic photoperiod		
	Winter eggs only	Winter then summer eggs	Summer eggs only	Summer then winter eggs	Total	Mean time interval to switch-over (days)	Mean no. eggs laid before switch-over	Total	Mean time interval before last egg (days)	Mean no. eggs laid
Protonymph	0	0	10	0	10	< 12	—	0	—	—
Deutonymph	0	0	10	0	10	< 8	—	0	—	—
Teneral ♀	3	7	0	0	7	15	15	3	22	22
Egg-laying ♀	6	4	0	0	4	10	18	6	16	23
Protonymph	10	0	0	0	10	< 12	—	0	—	—
Deutonymph	9	0	0	1	10	< 9	—	0	—	—
Teneral ♀	0	0	1	9	9	13	16	1	14	23
Egg-laying ♀	0	0	5	5	5	10	24	5	18	32

as a deutonymph from an 8 to a 16 hr. photoperiod the first eggs were of the summer type (Fig. 7A). And in the reciprocal transfer (Fig. 7B) nine mites behaved in a similar manner, their first eggs being of the winter type. Nevertheless, one female began by laying summer eggs and must therefore have already been influenced as a deutonymph by the initial long photoperiod.

This last mite deposited eighteen summer eggs during the first 10 days of reproductive life and then, in response to the antagonistic photoperiod, began abruptly to lay winter eggs and continued thereafter to do so until spent. Many further examples of partial or 'labile' determination, as revealed by the 'switch-over' in egg type, are given in the following paragraphs. The character of the eggs and the behaviour of the ovipositing females during the period of transition are considered in greater detail under a separate heading (p. 474).

At 15° C. newly emerged females feed for 3-4 days before depositing the first egg. When such teneral mites were transferred, the character of their first eggs was invariably determined by the conditions of photoperiod prior to transfer. It is

evident therefore that the process of determination proceeds actively during the deutonymphal instar. When these mites were exposed further to the antagonistic photoperiod a high proportion switched over to the alternative egg type. For example, seven presumptive winter females when transferred to a long photoperiod laid an average of fifteen winter eggs followed by summer eggs; while nine presumptive summer females transferred to a short photoperiod laid an average of sixteen summer eggs followed by winter eggs (Table 7). It will be noted, however, that a few mites were now completely impervious to the influence of the antagonistic photoperiod. Thus three presumptive winter females laid an average of twenty-two winter eggs each over a period of 22 days in spite of continued exposure to a long photoperiod; similarly, one presumptive summer female laid only summer eggs.

The results with older female mites which were only transferred to the second photoperiod after one to three eggs had been laid, show that the determination process is still reversible in some individuals. For example, four winter females responded to the long photoperiod, laying an average of eighteen winter eggs followed by summer eggs; and five summer females responded to the short photoperiod. However, a higher proportion of females (eleven out of twenty) were now resistant to the antagonistic photoperiod.

The progress of determination with advancing age is indicated by: (i) the length of the time lapse between exposure to the second photoperiod and the switch-over; (ii) by the proportion of individuals in which the switch-over occurs. On this basis, the deutonymphal instar is clearly the first and also the most sensitive stage in the life history. Development is switched in 8-9 days or possibly less (since this is the time required at 15° C. for the active deutonymph to develop into a female and for that female to lay the first egg). Moreover, every individual is influenced by the photoperiod during this stage of development. In teneral females the usual time lapse before switch-over was 13-15 days and only sixteen out of the total of twenty mites were thus affected. Evidently determination has proceeded further and the sensitivity is reduced correspondingly. Egg-laying females appear to be still less responsive since a switch-over occurred in only nine out of twenty individuals, although in these mites the time lapse did not differ greatly from that in teneral females (Table 7).

While it is apparent that fewer cycles of illumination are required to direct the development of the deutonymph into a definitive path than to switch over development in the adult, the sensitivity of the deutonymph may actually be greater than indicated. Mites were therefore exposed continuously to a long-day regime except as deutonymphs when they received between one and eight short-day cycles. The incidence of winter females (Table 8) showed that although the effect of four cycles of the diapause-inducing photoperiod was perceptible, diapause did not become universal until virtually the whole of the deutonymphal instar was spent in short days.

A consideration of the results with long and short photoperiods indicates that these stimuli are equal in intensity. The ease of reversal by an antagonistic photo-

period is also constant in any given developmental stage irrespective of whether determination is proceeding towards the 'winter' or 'summer' conditions. Evidently, the rates of determination are also alike.

TABLE 8. *The effect on diapause of exposing deutonymphs of Metatetranychus ulmi to one or more daily cycles with a diapause-inducing 8 hr. photoperiod*

(The mites otherwise reared continuously with a 16 hr. photoperiod. Temperature, 15° C.)

No. of short-day cycles	1	2	3	4	5	6	7	8
% winter females	0	0	0	7	30	45	48	85

Temperature

Mites were reared continuously with an 8 hr. photoperiod but were transferred at different stages of development from 15° C. to a diapause-preventing temperature (25° C.), or vice versa. The first temperature is in itself 'neutral' but is diapause-inducing in conjunction with this photoperiod. It may also be recalled that the higher temperature does not entirely suppress diapause; approximately 27% of winter females are expected if the mites experience this temperature and a long photoperiod throughout development.

Females transferred to 25 or to 15° C. as protonymphs laid only summer or winter eggs respectively (Table 9A, B) and were thus unaffected by the conditions prior to transfer. Of the deutonymphs moved to 25° C. (Table 9A) four had been influenced by the initial stimulus since, as females, their first eggs were of the winter type. All four subsequently switched over to summer eggs in response to the high temperature. In the reciprocal experiment (Table 9B) none of the mites transferred as deutonymphs to 15° C. had been affected by the higher temperature prior to transfer since they laid winter eggs only.

When teneral females were exposed to the high temperature eight laid winter eggs at first but soon switched over to summer eggs. Two mites began with summer eggs, indicating that the switch-over must have occurred in the 3-4 days prior to the deposition of the first egg. The reciprocal experiment with teneral females is less informative, for the five mites which laid only winter eggs were probably individuals which had remained unaffected by the high temperature during the deutonymphal instar. Two mites were resistant to the antagonistic conditions, laying only summer eggs; three switched over from summer to winter eggs.

These results show that the earlier instars are indifferent to temperature and that determination by a temperature stimulus, as by photoperiod, occurs during the deutonymphal instar.

Older females again display a greater resistance to an antagonistic stimulus than teneral females. Although eight winter females switched over to summer eggs in response to the high temperature, two continued to lay winter eggs until spent. The summer females developing at 25° C. were segregated from the smaller numbers

of winter females. When the former were exposed to the lower temperature five switched from summer to winter eggs and five laid only summer eggs.

TABLE 9. *The sensitivity of different developmental stages of Metatetranychus ulmi to temperature*

(Ten mites in each category were transferred from a diapause-neutral temperature (15° C.) to a diapause-preventing temperature (25° C.) (A), or vice versa (B). Photoperiod, 8 hr. throughout.)

	Stage transferred	No. of mites laying				Mites responding to the second and antagonistic temperature		Mites not responding to antagonistic temperature	
		Winter eggs only	Winter then summer eggs	Summer eggs only	Summer then winter eggs	Mean time interval to switch-over (days)	Mean no. eggs laid before switch-over	Mean time interval before last egg (days)	Mean no. eggs laid
A	Protonymph	0	0	10	0	< 6	—	—	—
	Deutonymph	0	4	6	0	< 5	—	—	—
	Teneral ♀	0	8	2	0	4	4	—	—
	Egg-laying ♀	2	8	0	0	8	10	14	22
B	Protonymph	10	0	0	0	< 12	—	—	—
	Deutonymph	10	0	0	0	< 8	—	—	—
	Teneral ♀	5	0	2	3	12	10	21	29
	Egg-laying ♀	0	0	5	5	11	16	18	31

A high temperature seems to constitute a more intense stimulus than a long photoperiod for the time lapse before switch-over is much shorter. In deutonymphs it is less than 5 days, in teneral females 4 days and in older females about 8 days.

When teneral mites and older summer females experienced the short photoperiod at 15° C. the average time lapse to switch-over was 12 and 11 days respectively (Table 9B). Summer females in which the original determining stimulus was a long photoperiod and not a high temperature behaved in very similar manner (cf. Table 7B). Evidently the response is independent of the initial determining stimulus.

Photoperiod and temperature in combination

As would be expected, this association of stimuli proved to be more active in preventing diapause than either long photoperiod or high temperature alone. Of the teneral females previously reared at 15° C., and with an 8 hr. photoperiod, nine laid winter eggs followed by summer eggs; in one mite development was even switched over to the summer condition before the first egg was laid (Table 10A). All the egg-laying winter females tested were also susceptible to a stimulus of this intensity. The average time lapse to switch-over was only 3 days in teneral females and 6 days in older females.

The behaviour of mites transferred in the reverse direction (Table 10 B) was the same as in previous experiments (Tables 7 B and 9 B) when the diapause-inducing conditions after transfer were identical. This again show that although the initial stimulus serves to direct development either towards the summer or winter condition, the facility with which this process is reversed is governed by the time factor in development and not by the intensity of the primary determining stimulus.

TABLE 10. *The response of Metatetranychus ulmi to a combination of stimuli, namely high temperature and long photoperiod, that is strongly diapause-preventing*

(Ten mites in each category were transferred from an 8 hr. photoperiod at a temperature of 15° C. to a 16 hr. photoperiod at 25° C. (A), or vice versa (B).)

		No. of mites laying				Mites responding to the antagonistic photoperiod and temperature		
		Winter eggs only	Winter then summer eggs	Summer eggs only	Summer then winter eggs	Total	Mean time interval to switch-over (days)	Mean no. eggs laid before switch-over
Stage transferred								
A	Protonymph	0	0	10	0	10	< 6	—
	Deutonymph	0	1	9	0	10	< 5	—
	Teneral ♀	0	9	1	0	10	3	3
	Egg-laying ♀	0	10	0	0	10	6	15
B	Protonymph	10	0	0	0	10	< 12	—
	Deutonymph	9	0	0	1	10	< 8	—
	Teneral ♀	0	0	0	10	10	11	8
	Egg-laying ♀	0	0	8	2	2	15	25

While there is evidence of progressive determination in the adult mite, the reproductive organs, even late in life, often remain sufficiently plastic to respond to an antagonistic stimulus of adequate intensity. By a suitable manipulation of the stimuli it is even possible to induce a second switch-over. One mite, for example, was caused to lay nine winter eggs, thirteen summer eggs and finally seven more winter eggs.

Nutrition

Mites were reared on highly bronzed seedlings (stages 5 and 6) and were moved either as deutonymphs or egg-laying females to undamaged seedlings. The conditions of temperature (25 or 15° C.) and photoperiod (16 hr.) were constant throughout the experiments.

Records of the first eggs deposited on the undamaged plants by the older females show that the bronzed seedlings were producing about 29% of winter females at 25° C. and 43% at 15° C. (Table 11 A, B). The deutonymphs tested must therefore have included some mites which would ultimately have become winter females if allowed to remain on the bronzed plants. As all the deutonymphs subsequently

became summer females when plentiful food supplies were restored, the nutritional stimulus cannot yet have exerted a decisive determining influence. All the winter females transferred to undamaged seedlings at 25° C. switched over to summer eggs after the expected time lapse of 3 days. At 15° about half did so after an average time lapse of 10 days; the remaining winter females were completely resistant and laid an average of twenty-one winter eggs during 19 days of reproductive life.

TABLE II. *The diapause response of deutonymphs and females of Metatetranychus ulmi to the restoration of a plentiful food supply*

(The mites were transferred from highly bronzed to undamaged apple seedlings at 25° C. (A) or 15° C. (B). Photoperiod, 16 hr. throughout.)

	Stage transferred	No. tested	No. of mites laying			Mites responding to the nutritional factor	
			Winter eggs only	Winter then summer eggs	Summer eggs only	Mean time interval to switch-over (days)	Mean no. eggs laid before switch-over
A	Deutonymph	15	0	0	15	<5	—
	Egg-laying ♀	21	0	6	15	3	3
B	Deutonymph	15	0	1	14	<8	1
	Egg-laying ♀	35	8	7	20	10	7

As the sensitivity to the restoration of abundant food is clearly greatest during the deutonymphal instar, it is probable that deutonymphs are also particularly sensitive to restriction of the food supplies.

The character of the eggs during the period of transition

Certain abnormalities in the eggs are sometimes apparent during the period when the reproductive organs are undergoing a functional reversal; and errors in the choice of oviposition site also occur with abnormal frequency. The principal differences between winter and summer eggs, which have already been described (p. 451), include slight differences in size. In the following paragraphs measurements of egg size are given in terms of arbitrary units of a micrometer eyepiece. The mean diameter of summer eggs was 36 units and of winter eggs 41 units.

In many instances the change in egg type is abrupt and the oviposition behaviour perfectly in harmony with the character of the eggs. The last egg before the switch-over is then a completely normal winter or summer egg which is laid in the appropriate position on bark or leaf, while the first and subsequent eggs after switch-over are equally normal eggs of the opposite type. For example, a summer female exposed to a diapause-inducing photoperiod at 15° C. laid three lightly pigmented summer eggs on leaves (39, 37, 38 units) followed by a succession of normal deeply pigmented winter eggs on the bark (diameters of first three: 40, 40, 40 units).

When disturbances occur they are usually confined to a few eggs, often less than six. In the following list of abnormalities the direction of transition was from winter to summer eggs: (i) The eggs may be of intermediate size. For example, a mite laid a sequence of winter eggs on the bark (43, 42, 40, 42, 40, 41, 39). The last egg was of normal winter pigmentation but hatched after 8 days. There followed a sequence of normal summer eggs laid on leaves (37, 36, 38, 37 etc.). (ii) The previous example also illustrates the fact that eggs, which from their pigmentation and often from their size also, would be classed as 'winter', nevertheless develop without diapause. Sometimes these eggs are distributed irregularly in an otherwise normal sequence of diapause eggs. For example, a winter female during the period of transition laid the following sequence of eggs on the stem of the plant: three normal winter eggs (42, 40, 40), one deeply pigmented egg (40) which hatched, one normal winter egg (40), one deeply pigmented egg (40) which hatched, three normal winter eggs (40, 41, 41). The next eggs were all normal summer eggs (37, 36, etc.) laid on the leaves. (iii) There are unusually frequent 'errors' in the selection of the oviposition site. Thus the last winter eggs before the switch-over are often found on the leaves but are otherwise normal in size, pigmentation and diapause character. (iv) The first eggs after the switch-over may be deposited on the stem, although in all other respects they appear to be of the normal summer type. This behaviour is extremely rare with normal uncrowded summer females (see p. 452).

Reversals from summer to winter eggs often involve similar aberrations. The last summer eggs on the leaves may be unusually large, although they are lightly pigmented and hatch without diapause; or they may be both large and deeply pigmented. On the other hand, the first diapause winter eggs may be rather small or lightly pigmented; and they may be laid on the leaves.

It will be apparent that in 'intermediate' eggs the four characters which have been considered are associated in ways atypical of either summer or winter eggs. Nevertheless, the manner of association is apparently random and is independent of the direction of the switch-over. It seems that the processes determining these characters must also be subject to slight random variations in rate.

TERMINATION OF DIAPAUSE IN *METATETRANYCHUS ULMI*

The winter eggs enter diapause soon after the formation of the blastoderm. Once growth has ceased the embryos invariably fail to develop beyond this stage when maintained at a high temperature (e.g. 25° C.) and ultimately die without hatching. Exposure to low temperatures within the range that would normally occur during the winter months is effective in bringing diapause to an end. This seems to be the only important environmental agency involved.

Apple shoots thickly encrusted with winter eggs were obtained from an Essex orchard in mid-October, that is, before the onset of cold weather.* The shoots,

* The writer is indebted to Mr M. D. Austin for supplying this material.

when cut into convenient lengths, were immediately stored in darkness at constant temperatures of -5 , $1-2$, 5 , 9 and 18°C . After chilling had been in progress for periods extending up to 300 days, eggs were placed in a dark incubator at 25°C . for hatching. If the treatment has been effective larvae begin to emerge after a 9-day incubation period, and hatching then continues on a diminishing scale for some 10 or more days afterwards, finally ceasing completely after about 25 days.

TABLE 12. *Termination of diapause in the winter eggs of Metatetranychus ulmi by exposure to low temperatures*

(Average number of eggs per experiment, 970.)

Date and year collected	Temp. ($^{\circ}\text{C}$.)	Percentage eggs hatching at 25°C . after exposure to lower temperature for					
		20 days	50 days	100 days	150 days	200 days	300 days
20 Oct. 1948	$1-2$	0	0	7	13	68	29
	5	0	0.3	21	—	57	11
	9	0	0.2	15	—	62	—
	18	0	0	0	0	0	0
18 Oct. 1949	-5	0	0	0.7	0	0	—
	$1-2$	0	0	15	50	78	13

The results of experiments using material collected during 1949 and 1950 are given in Table 12. If the rate of termination of diapause is regarded as indicating the effectiveness of the chilling treatment, almost equal activity is shown by temperatures of 1 , 5 and 9°C . Exposure to a temperature of 18°C ., even for very long periods, does not bring diapause to an end. Freezing the eggs at -5°C . also fails to break diapause and results in heavy mortality after about 100 days. The upper limits of the reaction therefore lie between 10 and 18°C . and the lower between $+1$ and -5°C .

The complete arrest of growth at high temperatures and the protracted nature of the response at low temperatures both indicate that diapause in *M. ulmi* is relatively intense. Virtually no hatching occurs after chilling at $1-2$, 5 , or 9°C . for 50 days, and about 200 days are needed for the percentage hatch to reach the recorded maxima of 57–78%.

The temperature threshold for post-diapause development is approximately 7°C . After diapause has been terminated, the embryos can still survive at a subthreshold temperature for a limited period. The reduction in the numbers hatching after 300 days at $1-2$ or 5°C . suggests, however, that the majority of the embryos have succumbed during the preceding 100 days. The temperature range of the processes responsible for terminating diapause and for embryonic development overlap at 9°C . Winter eggs exposed continuously to this temperature began to hatch after about 160 days and 62% had hatched by 200 days.

Since the winter eggs respond to temperatures as high as 9° C. the date of collection is of considerable importance when eggs laid in the field are used for experimental purposes. It is even possible that by mid-October some 'diapause development' (Andrewartha, 1952) may have occurred. Winter eggs laid only 2 weeks previously under known conditions in the laboratory (15° C. and short photoperiod) were therefore chilled at 2° C. for 150 days. 33% hatched when subsequently incubated at 25° C. After identical treatment 50% of the eggs collected in 1949 and 13% of those collected in 1948 subsequently hatched. This suggests that by mid-October the eggs have not been materially affected by previous temperature conditions.

Dierick (1950) has stated that winter eggs collected at the end of November in Holland hatch during two periods, the first extending from 9 to 22 days and the second from 40 to 80 days after the beginning of incubation at 20° C. By this calendar date the eggs will already have been exposed to an effective period of chilling, and this may possibly account for hatching during the 'first period'. In the present experiment no hatching during a subsequent 'second period' was observed. If diapause development was incomplete, the eggs always failed to hatch even after prolonged exposure to a high temperature.

Dierick has further recorded that the proportion of eggs hatching during the 'first period' is augmented if they are first dipped in xylene for 5–20 min. This finding is compared with Slifer's (1946) observations on the breaking of diapause in the egg of *Melanoplus differentialis* by treatment with xylene. Dierick concludes that the removal of a waxy layer from the shell—a process which is hastened by solvent action—may be necessary prerequisite for the termination of diapause. Although such waxy layers are known to exist in the shell of the red spider mite (Beament, 1951) the analogy with the *Melanoplus* egg may nevertheless be rather remote. So far as is known, no water absorption takes place in the winter egg prior to the resumption of growth. An alternative suggestion would be that the penetration of the solvent through the shell in subtoxic quantities serves as a stimulant to embryonic development.

Light does not appear to influence the termination of diapause in *M. ulmi*. Eggs incubated continuously at 25° C. failed to hatch either in darkness or when subjected over many weeks to a long photoperiod. Eggs in which diapause had been terminated by chilling also appeared to hatch normally in darkness. However, detailed comparisons by Hueck (1951) of the hatching of such eggs in light and darkness have shown that a proportion, sometimes as high as 23%, fail to hatch in the dark, although the embryos are fully developed. Hueck concludes that light may stimulate the larva to break the shell. Although few unhatched eggs with fully developed embryos were noted in the present experiments it may be that the chilling treatment was somewhat more effective than the incidence of hatching, as recorded in Table 12, would indicate.

DIAPAUSE IN OTHER SPECIES OF TETRANYCHIDAE

Tetranychus telarius L.

In the common glasshouse red spider mite diapause occurs in the adult. Female mites of the summer generations vary in colour from pale green to straw yellow and lay pearly white eggs which develop without diapause. Females that are destined to enter diapause in late summer are at first normally coloured, but after a few days cease to feed and begin to turn pink. One week afterwards they have assumed the bright scarlet pigmentation characteristic of the overwintering form.

These mites then leave the plants, often hanging in vast numbers from the webs of silk which festoon badly infested vegetation. They may show considerable activity if the temperature is sufficiently high but as long as diapause persists they refuse to feed. In the spring the overwintered mites return to the host plants. The scarlet pigmentation is then gradually dissipated as they begin first to feed and then to lay eggs. Although the significance of these pigmentary changes is unknown, it is interesting that they should be paralleled by the assumption of the deeper red pigmentation in the winter egg of *Metatetranychus ulmi*.

For experimental work cultures were started with mites collected in summer from a Cambridge glasshouse. Apple seedlings were again used as the host plants and these were maintained under diapause-preventing conditions (high temperature and long day). Diapause is facultative. As in *M. ulmi*, the incidence of diapause in any given generation is in no way affected by the number of successive non-diapause generations through which the mites have previously passed.

Induction of diapause. The environmental factors which govern the onset of diapause in *M. ulmi* have the same significance in *Tetranychus telarius*.

TABLE 13. *The influence of photoperiod and temperature on the percentage of females of Tetranychus telarius entering diapause*

(Average number of mites per experiment, 47.)

Temp. (° C.)	Photoperiod (hr./24 hr.)						
	0	4	8	12	14	16	24
15	18	100	100	100	13	0	0
25	0	2	31	45	—	0	0

Photoperiod. The response to daily photoperiods of different duration is shown in Table 13. With photoperiods extending from 4 to 12 hr., and at a temperature of 15° C., every female enters diapause. The incidence of diapause declines rapidly with photoperiods longer than 12 hr. and is completely lacking at 16 hr. and in uninterrupted illumination. The transition from summer to winter forms is most rapid at a critical photoperiod of 12-14 hr. Diapause again falls (to only 18%) in the absence of light.

Temperature. High temperatures (e.g. 15° C.) tend to prevent diapause, as in *Metatetranychus ulmi*. (Table 13).

Nutrition. Heavily infested seedlings were kept under long day conditions at 15 or 25° C. until the foliage became severely bronzed. At the higher temperature rapid dispersal took place so that the plants were ultimately left devoid of mites. No females in diapause were recovered. However, the tendency to migrate was apparently less at the lower temperature for a variable proportion of females entered diapause while still on the plants. Exhaustion of the food supplies seems therefore to induce diapause, as in *M. ulmi*.

The termination of diapause. The state of diapause can be broken by chilling the mites. Large numbers of red overwintering females were collected in early October from infested maize plants growing in a Cambridge glasshouse. Batches averaging forty-one mites were stored at constant temperatures of 25, 19, 10 and 1° C. in a saturated atmosphere. These were brought out at intervals and placed on fresh apple foliage at 25° C.

TABLE 14. *Termination of diapause in overwintering females of Tetranychus telarius, by exposure to low temperatures*

(Average number of mites per experiment, 41.)

Temp. (° C.)	Percentage females which resumed feeding at 25° C. after exposure to lower temperatures for		
	50 days	75 days	100 days
1	3	85	97
10	1.5	44	87
19	0	0	—

At 25° C. only 18% of the mites remained alive after 50 days but all of these retained the red body coloration and refused to feed. Neither was diapause terminated at 19° C., although 10% remained alive after 75 days (Table 14). However, temperatures of 10 or 1° C. proved effective, the chilled mites readily beginning to feed after they had been exposed to warmer conditions for a few hours. Diapause was partially terminated after 75 days and was completely terminated by 100 days. The range of effective temperatures is thus the same as in *M. ulmi* but the intensity of diapause is somewhat less.

Metatetranychus bioculatus Wood-Mason

Living material of *M. bioculatus* was received from the Tocklai Experimental Station, Assam, through the courtesy of Mr E. H. Hainsworth. The geographical distribution of this mite, the common red spider mite of tea, is known to include Assam and southern India. This range is therefore likely to include areas where the

seasonal variations in day-length are slight or even absent. Earlier studies of the life history in Assam (Andrews, 1928) have shown that small breeding colonies occur on the tea bushes during the cool season, but whether hibernating stages are also present has not finally been established.

Stock cultures were reared on cut *Camellia* shoots and then on apple seedlings which proved to be equally satisfactory. The mites have a strong preference for the upper, illuminated surface of the foliage where they feed in relatively compact colonies. The damage caused by the feeding punctures (a bronzing which is very similar in appearance to that produced by *Metatetranychus ulmi*) is at first confined to the region of the veins and mid-rib but spreads gradually over the whole leaf surface as the size of the colony increases. The eggs, which are laid in these areas of the leaf, hatched within a few days when the cultures were kept under long day conditions at 25° C.

Mites were reared on normal undamaged apple foliage at 15° C. and with photoperiods of 4 or 12 hr.—conditions which would be strongly diapause-inducing in *M. ulmi*. The females continued, nevertheless, to deposit non-diapause eggs. Similarly, when seedlings were allowed to become overpopulated and highly bronzed at temperatures either of 15 or 25° C. the mites migrated from the leaves on to the stems but no diapausing stage appeared. There seems little doubt that this species is without diapause and the question of a response to photoperiod does not therefore arise.

DISCUSSION

The phenology of Metatetranychus ulmi

The life cycle of *M. ulmi* in southern England, as represented in Fig. 6, is based on the comprehensive studies of Blair & Groves (1952). There are five overlapping generations annually. The time relations depicted are the average for three years (1947 to 1949).

Since all developing stages prior to the deutonymph are insensitive to the environmental agencies which govern diapause, and since maximum sensitivity also occurs during this instar, the dates when the first deutonymphs of each generation appear and when the last deutonymphs have developed into adults, are of considerable phenological significance. In Fig. 6 these have been marked by vertical bars.

The seasonal photoperiod appropriate for *M. ulmi* is also shown in Fig. 6. As the threshold of illumination is no more than 1–2 f.c., the effective day-length must include periods of twilight after sunset and before dawn. In the absence of systematic measurements under English conditions, figures compiled at the Potsdam observatory (situated on the same parallel of latitude, namely 52° N.) have been taken as a rough guide (Israël, 1941). Assuming 2 f.c. to be the limiting light intensity, there was a daily average of 25–35 min. of photoperiodically active dusk twilight during the months of January to October 1939, and 25 min. of effective dawn twilight from December to March. In constructing the curve in Fig. 6, 1 hr.

has therefore been added at all dates to the day-length from sunrise to sunset. The incidence of diapause expected at 15° C. with corresponding photoperiods is also drawn in Fig. 6.

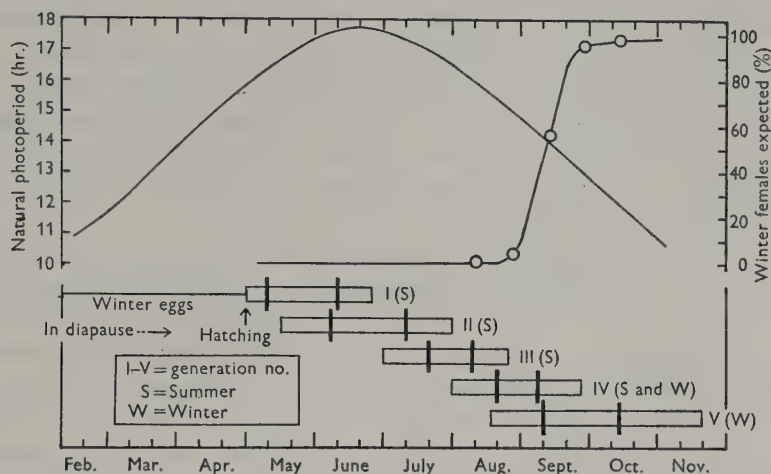


Fig. 6. The life cycle of *M. ulmi* in England in relation to the natural photoperiod.

The course of the life cycle may now be considered. In the field the diapause condition of the winter eggs is not completely terminated until mid-March, although some eggs will hatch when brought into warm conditions during December (Blair & Groves, 1952). The eggs normally begin to hatch during the third week in April, and have thus been exposed to autumn and winter temperatures for some 5-6 months (October to March). This is very close to the time required (*c.* 150 days) to terminate diapause experimentally by chilling the eggs at constant temperatures. It does not therefore seem necessary to assume that fluctuating temperatures play any special role in terminating diapause in nature. Maximum effectiveness is shown by any temperature between +1 and 9° C., and the range may even extend higher in the temperature scale. It may then be that the winter eggs are exposed almost continuously during the dormant season to temperatures which, although fluctuating, fall within this range of effectiveness.

The long obligatory period of diapause serves to delay hatching until there is foliage available for the first generation mites. And this timing also ensures that the first deutonymphs do not appear until the middle of May. As the photoperiod has by then lengthened to 16 hr. all the 1st generation mites become summer females.

When the mite populations on the host plant remain consistently small throughout the season, so that the foliage is little damaged, the photoperiod probably plays a major role in determining the proportion of winter and summer females in each

generation and hence the generation number also. This response certainly accounts for the appearance of winter females at a time when the food supplies are still plentiful.

It is apparent from a consideration of the photoperiod (Fig. 6) that the first three summer generations would be expected to comprise only summer females. The last deutonymphs of generation III appear by about 10 August when the photoperiod, although now decreasing, is still nearly 16 hr. By the third week in August the photoperiod has fallen to 15 hr. and by 10 September to 14 hr. These dates correspond approximately with the time of appearance of the first and last deutonymphs of generation IV. Under experimental conditions at 15° C. these photoperiods produced 4 and 54% of winter females respectively. As day-length clearly becomes critical at this season generation IV would be expected to include both summer and winter females, and the first winter females should appear at the end of August. In the 5th generation the first females to develop might still include some summer females; but after 25 September, when the photoperiod has decreased to 13 hr., the proportion of winter females would be expected to exceed 90%.

The temperature factor will no doubt influence diapause but it is likely to be less decisive than the photoperiod. It will be recalled that temperature appears to influence diapause principally during the dark phase; hence it is the night temperature which is most relevant in this connexion. Without resorting to detailed analyses of actual records, a temperature of 15° C. represents a fair approximation to the mean night temperature from June to September in southern England. Should higher night temperatures occur during the summer months, this factor, in augmenting long photoperiod, would tend to prevent the occurrence of diapause in the first three generations. During October when the night temperature often falls below 10° C. this factor will reinforce the action of the short photoperiod in inducing diapause in the fifth generation mites.

The role of nutrition would also seem to be subsidiary if foliage bronzing remains slight throughout the season. In these circumstances nutrition will exert no influence on diapause until late September and October when the apple foliage begins to undergo senescence. This condition will then favour the production of winter females and will further reinforce the action of photoperiod and temperature.

This interpretation is in conformity with the course of the life cycle as described by Blair & Groves (1952). In slightly damaged orchards where little leaf fall has occurred by mid-September, the first three annual generations are composed only of summer females. Winter females develop in the 4th generation and the first winter eggs appear on the trees about the third week in August. In commercial plantations, however, it is difficult to be certain that there has been no significant reduction in the available food supplies, even though little bronzing is visible. Corresponding records of the deposition of winter eggs in unsprayed orchards harbouring very small mite populations would provide a more accurate guide to the time of operation of the critical photoperiod.

The 5th generation in the field consists essentially of winter females. The few summer eggs still present on the leaves in September (Blair & Groves, 1952, Fig. 4) may have been laid by the first females of this generation to develop. These eggs yield only an incomplete 6th generation since these mites cannot reach maturity before leaf fall.

The observations of Kuenen (1946), Blair (1951) and Blair & Groves (1952) on the life cycle in sprayed orchards have shown that this pattern of the life cycle may be greatly modified if the mite population reaches outbreak proportions. When severe bronzing occurs early in the season some winter females may develop prematurely in the 3rd generation and even in the 2nd if the damage is of particular severity. In such instances the nutritional factor has assumed a dominant role. The present studies have shown that partial exhaustion of the food supplies can entirely annul the influence of the long day-length which would otherwise prevent diapause at this season.

Reference may now be made to certain more general aspects of phenology, particularly to the length of the reproductive season as affecting the annual generation number and to the co-ordination displayed in the responses to factors which terminate and evoke diapause.

The great proliferation of red spider mites under orchard conditions is often considered to be due to the destruction of their predators. However, as population unbalance of this kind is likely to be rare in nature, the exhaustion of the food supplies and the consequent premature deposition of winter eggs may also be somewhat artificial phenomena. If these circumstances are excepted, the duration of the reproductive season is seen to be relatively constant since it is stabilized by the long obligatory period of diapause and by the action of photoperiod. On the other hand, the generation number, either from year to year or from one locality to another will be less stable since, in any given latitude, this will be dependent upon conditions, particularly those of temperature, which are inherently variable. For example, in the Pacific North-West of America *M. ulmi* also begins to lay winter eggs in mid-August, no doubt in response to photoperiod (the latitude is similar). But although the reproductive season is no longer than in England, the higher summer temperatures permit seven generations to develop annually (Newcomer & Yothers, 1929).

It follows that the incidence of winter females in the first diapause generation will also be a variable feature. Comparatively slight difference in temperature will, by accelerating or retarding development, alter the proportion of mites which has passed through the sensitive deutonymphal stage at the time when photoperiod becomes critical.

In *M. ulmi* and *Tetranychus telarius* 'diapause development' and the day-length response are so attuned that photoperiod will normally prevent the recurrence of diapause in the first summer generation. However, this co-ordination has been observed to break down when the latter species is exposed to semi-artificial conditions. The behaviour of this mite in unheated glasshouses is as follows. The

overwintering females, as a result of prolonged chilling, emerge from diapause in April and start to feed actively. Many generations are produced during the summer months, and females again begin to enter diapause in September in response to reduced day-length. If the temperature is low few active mites remain by November, even though the food supplies may still be plentiful.

In tomato and cucumber houses which are not heated until they are planted up in January, some diapausing mites immediately begin to feed and lay eggs. But the 1st generation mites again turn red and re-enter diapause (Miles & Miles, 1948, p. 151). Under these conditions the period of chilling has been adequate to terminate diapause in some of the overwintering females, but in February or early March the photoperiod is still sufficiently short to induce diapause for a second time.

The adaptive significance of diapause and of the response to photoperiod

Since *Metatetranychus ulmi* feeds almost exclusively upon the leaves of deciduous host plants, the general adaptive value of diapause can scarcely be questioned. At the same time it is also clearly advantageous that in such a rapidly breeding species diapause should be of the facultative type. The reproductive potential will then be increased in proportion to the generation number. The selective advantage of an obligate diapause only becomes manifest in more slowly reproducing arthropods when the length of the favourable season is insufficient for the maturation of two complete generations.

The ability to respond to three distinct environmental factors when any one might appear adequate to induce dormancy in autumn is a notable feature of the diapause behaviour of *M. ulmi*. Although the length of the reproductive season is, in general, limited by photoperiod, the temperature response undoubtedly gives the life cycle additional flexibility which might prove of value in other parts of the geographical range. The reproductive season could be extended, even under short day conditions, if temperatures were high enough to prevent diapause.

The response to reduction of the food supply may have evolved in relation to the cycle of growth and senescence in the host plants. If the available food is exhausted by the feeding activities of the mites themselves, diapause is induced in a similar manner; however, the latter circumstances seem to be incidental rather than primary since mite populations of the size required to cause extensive bronzing of the host plants seem only to occur under orchard conditions when population balance and other conditions have been grossly disturbed.

As the turn-over to winter forms can be caused by foliage senescence alone, the value of the day-length response may legitimately be questioned. A fact of some possible significance is that oviposition in *M. ulmi* is a relatively slow process, since the mite must feed for an appreciable period before each egg is matured. Yet the foliage of deciduous trees undergoes senescence rather rapidly and in its entirety, then becoming worthless as food. If diapause were evoked only by the imminent

stimulus of nutrition many mites would be prevented from laying their full complement of eggs. The photoperiodic response, which ensures that a large proportion of the winter eggs are laid *before* adverse conditions set in, should allow a larger winter egg population to be built up.

Certain general aspects of photoperiodism also require comment.

(i) Many arthropods from the north temperate regions which respond to short days enter diapause before the September equinox. Hence it would not be surprising if species from low latitudes were found to respond to a shorter critical photoperiod than do those from higher latitudes. Similarly, intra-specific differences of a comparable nature might also occur in species with a wide geographical range.

There is some evidence that geographical strains of *Tetranychus telarius* do differ in this respect. In the Cambridge (52° N.) strain used in the present experiments the turn-over to winter forms was most rapid at 13–14 hr. According to Bondarenko (1950), the critical photoperiod in a strain from Leningrad (60° N.) was 16 hr. 40 min. If no allowance is made for temperature and for the probable influence of photoperiodically active twilight, the Cambridge population would be expected to enter diapause at the beginning of September, the Leningrad population at the beginning of August.

(ii) The direction of the response to photoperiod is a further provision for ensuring entry into diapause at the appropriate season. The time interval separating the perception of the photoperiodic stimulus and the onset of diapause appears to be the decisive factor. When, as in Tetranychid mites, the light-sensitive stage immediately precedes the diapausing stage, and when the species also possesses a high intrinsic rate of development, the response quickly follows the stimulus. In these and in many 'long day' insects (see Danilyevsky & Gayspitz, 1948) a long photoperiod prevents and a short photoperiod induces diapause. These conditions are reversed in a 'short-day' insect such as the common silkworm (Kogure, 1933). As the eggs of *Bombyx mori* show the greatest sensitivity to light, the translation of the stimulus requires a time interval equal to the period of development of an entire generation. However, the direction of the response is such that eggs exposed to the long days of summer will eventually yield moths which lay eggs of the appropriate diapause type in autumn. The temperature response is also reversed. High summer temperatures will therefore also tend to induce diapause in the subsequent generation.

(iii) If day-length is to be a reliable indicator of season it is clearly desirable that the response should not be influenced by random fluctuations in light intensity. In all the arthropods so far examined the response has in fact proved to be independent of light intensity, provided a certain threshold is exceeded. In *Metatetranychus ulmi* the threshold is too high for the mite to be affected by bright moonlight.

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THE SIGNIFICANCE OF THE LIGHT AND DARK PHASES IN THE PHOTOPERIODIC CONTROL OF DIAPAUSE IN *METATETRANYCHUS ULMI* KOCH

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(With 2 Text-figures)

The daily cycle of illumination is one of several agencies which control the onset of diapause in *Metatetranychus ulmi*. Both light and dark phases in the cycle are concerned in the determination process.

In general, a long light phase tends to suppress and a long dark phase to induce a diapause. In any combination, the path of development is decided by the balance between diapause-preventing (light phase) and diapause-inducing (dark phase) stimuli. However, as their effectiveness does not increase linearly with duration, the existing balance changes with the phase duration.

The effectiveness of the light phase in suppressing diapause increases most rapidly between 8 and 16 hr.; that of the dark phase rises very sharply between 8 and 12 hr. Longer dark periods of up to several days duration also induce diapause but are no more effective than a 12 hr. phase. The inclusion in the cycle of very long periods of light or darkness may also influence diapause by reducing the number of complementary phases experienced by the mite during the sensitive period of development.

M. ulmi is highly insensitive to the interruption of effective light and dark phases by short intervals of darkness or light—a further indication of the slow inception of the light- and dark-phase reactions.

These findings are discussed in terms of hypothetical mechanism involving cumulative synthesis and removal of some active substance, but the experimental results cannot yet be fully reconciled with a simple hypothesis of this kind.

INTRODUCTION

In discussing the photoperiodic control of flowering in plants, Borthwick, Parker & Hendricks (1950) have emphasized that this term is something of a misnomer, since it is now well established that the intervening dark phase in the cycle of illumination plays at least as significant a role as the photoperiod in suppressing or inducing the formation of flower primordia. Recent work on the control of diapause in Lepidoptera by environmental factors has shown that in many species the occurrence of diapause is also determined by the hours of daylight which the insects experience during some sensitive stage of development. Unless the duration of the light and dark phases is varied independently, however, no decision can be reached as to whether the insects are responding to light or darkness or indeed to both. Only two species, namely the oriental fruit moth *Grapholitha molesta* (Tortricidae) (Dickson, 1949) and *Acronycta rumicis* (Agrotidae) (Danilyevsky & Glinyanaya, 1949, 1950)

seem to have been used in this type of experiment. Nevertheless, in these insects, as in plants, both light and dark components have been found to be critical.

In the fruit tree red spider mite the daily photoperiod is also one of several agencies governing the development of 'summer' and 'winter' females (Lees, 1953). The former lay non-diapause eggs, the latter diapause. The present paper shows that *Metatetranychus ulmi* resembles the two species of Lepidoptera in responding both to the light and dark phases in the cycle of illumination. But the entirely different behaviour in the presence of certain identical regimes of light and darkness suggests that there are fundamental dissimilarities in the mechanism of the photoperiodic reaction.

METHODS AND MATERIALS

The mites were reared on small potted apple seedlings at 15° C., as described in the previous paper (Lees, 1953). The influence of photoperiod is particularly clearly defined at this medium temperature. With a 'long day' of 16 hr. and a 'short night' of 8 hr. no diapause occurs; with a 'short day' of 12 or 8 hr. and a complementary 'long night' of 12 or 16 hr. diapause is almost universal.

The diapause character of the egg is determined by the female mite. The incidence of diapause has therefore been expressed as the percentage of winter females. As the identity of a given mite can only be established by noting the type of eggs laid, it was necessary to observe each mite individually. Fifteen to twenty-five females were examined in each experiment.

'Natural' fluorescent tubes (2 ft., 20 w.) were employed as the light sources, the intensity of the illumination falling on the upper surface of the plants being in the region of 100 f.c. If the spectral energy distribution of the source is continuous, as with 'white' light emitted by a fluorescent or tungsten filament lamp, diapause in *M. ulmi* is affected only by the periodicity of the illumination, and is independent of intensity. The precise illumination is therefore immaterial, provided the intensity exceeds a threshold of about 2 f.c. (Lees, 1953).

The duration of the light and dark periods was controlled independently by a pin-wheel timing device with automatic re-setting which enabled any desired cycle between 2 and 48 hr. to be obtained. The writer is greatly indebted to Dr R. H. J. Brown of this Department for the design and construction of this instrument.

THE LIGHT PHASE

When the duration of the dark phase is held constant and the light phase progressively lengthened, the incidence of diapause falls in a systematic manner (Table 1). Long intervals of light therefore tend to suppress diapause.

This is clearly seen if the dark phase is short. Diapause then declines progressively as the photoperiod is extended from 4 to 8 hr., from 8 to 12 hr. and finally from 12 to 16 hr. With a constant dark phase of 4 or 8 hr. the effectiveness of the photoperiod in preventing diapause increases most rapidly between 8 and 12 hr.

(the proportion of winter females falling from 62 to 5% and from 96 to 10% respectively).

Photoperiods longer than 16 hr. also completely prevent diapause if the dark phase lasts for 8 hr. or less. With the extension of the dark interval to 12 hr. the diapause-inducing activity of the dark phase is greatly augmented (see below), and this factor then becomes dominant in most combinations. Nevertheless, it is probable that the role of light in suppressing diapause can still be detected even with dark phases of 12 or 24 hr. provided the photoperiod is sufficiently long. Thus a 60 hr. photoperiod is more effective in this respect than a 36 hr. photoperiod (Table 1).

TABLE 1. *The influence of different cycles of light and darkness at 15° C. on the incidence of diapause in Metatetranychus ulmi*

Cycle of illumination			Cycle of illumination		
Hours of light	Hours of darkness	% winter females	Hours of light	Hours of darkness	% winter females
Continuous	0	0	4	24	95
4	2	63	8	24	100
8	4	62	12	24	100
12	4	5	16	24	100
16	4	0	24	24	100
24	4	0	36	24	100
4	8	95	60	24	83
8	8	96	16	48	100
12	8	10	16	96	96
16	8	0	16	144	85
24	8	0	16	192	42
4	12	92			
8	12	96	0	Continuous	60
12	12	97			
16	12	95			
24	12	100			
36	12	78			
60	12	20			

However, no conclusive explanation of the partial prevention of diapause by very extended photoperiods can be offered for the following reason. Apart from the likely effect of photoperiod itself, it has to be borne in mind that the number of diapause-inducing dark phases (see below) which fall within the sensitive period of each developing mite is reduced, and these dark phases are also more widely separated in time. At a temperature of 15° C. the sensitive period continues for about 8 days, that is, from early in the deutonymphal instar to the time of deposition of the first egg (Lees, 1953). With a cycle of 36 hr. light and 12 hr. darkness, for example, the mites would experience a maximum number of four effective dark phases superimposed, in effect, on a background of continuous illumination. And if the photoperiod were lengthened sufficiently, virtually the whole of the sensitive

period would be spent in the light and no diapause could be expected. A previous experiment has shown that when the number of diapause-inducing (24 hr.) cycles falling within the sensitive period is reduced from eight to four, the incidence of diapause declines from 85 to 7% (Lees, 1953). Although the dark phases were given on consecutive days in this experiment and were not distributed over the whole sensitive period, the results provide grounds for believing that diapause will be strongly influenced by the number of effective dark phases.

On the other hand, there is some additional evidence suggesting that continuous illumination (and presumably extended photoperiods also) may prevent diapause more strongly than a 'long day' photoperiod of 16 hr. Mites developing at a low temperature (10° C.) enter diapause at nearly all photoperiods (Lees, 1953). Yet although one half of the mites reared at this temperature with a 16 hr. photoperiod proved to be winter females, no females of this type appeared when the illumination was continuous.

It seems that the progressive fall in the incidence of diapause with photoperiods of 24, 36, and 60 hr. should probably be attributed both to the increased photoperiod itself and to the reduction in the number of dark phases.

THE DARK PHASE

The dark phase influences the course of development in the direction of the diapause condition and therefore functions in opposition to the light phase. In general, then, diapause is induced more actively by long than by short dark intervals. Very extended dark phases, approximating in length to the duration of the sensitive period, provide the only exceptions to this rule.

The results contained in Table 1 have been rearranged in Fig. 1 so as to illustrate the role of the dark phase. Short dark periods of 4 and 8 hr. only favour diapause when combined with a correspondingly short light phase and are partially or wholly overcome by a 16 hr. or even a 12 hr. photoperiod. However, a further extension by 4 hr. is critical, for a 12 hr. dark phase completely annuls the influence of photoperiods as long as 24 hr. Even with a 60 hr. photoperiod 20% of winter females were still produced. A longer dark phase of 24 hr. duration seems to promote diapause slightly more strongly, as may be judged by its incidence in combination with photoperiods of 36 and 60 hr.; but the gain in effectiveness is much less than when the dark phase is extended from 8 to 12 hr.

The influence of very long dark intervals in combination with a 16 hr. photoperiod is also shown in Table 1. With dark intervals extending up to 4 days diapause was virtually universal, but 15% summer females developed with a dark phase of 6 days and 58% with one of 8 days. The progressive reduction in the number of long photoperiods falling with the sensitive period of each developing mite cannot account for the decline in diapause as this trend should, if anything, favour the diapause condition. It is probable that with dark intervals lasting for 6 and 8 days the mites are responding entirely to the dark phase (only one photoperiod could be

experienced during the sensitive period). The incidence of diapause with an 8-day dark interval does in fact approximate to that obtained in the complete absence of light.

The role of light and darkness may be summarized as follows. Provided the cycle of illumination is short in relation to the sensitive period, the light phase tends to

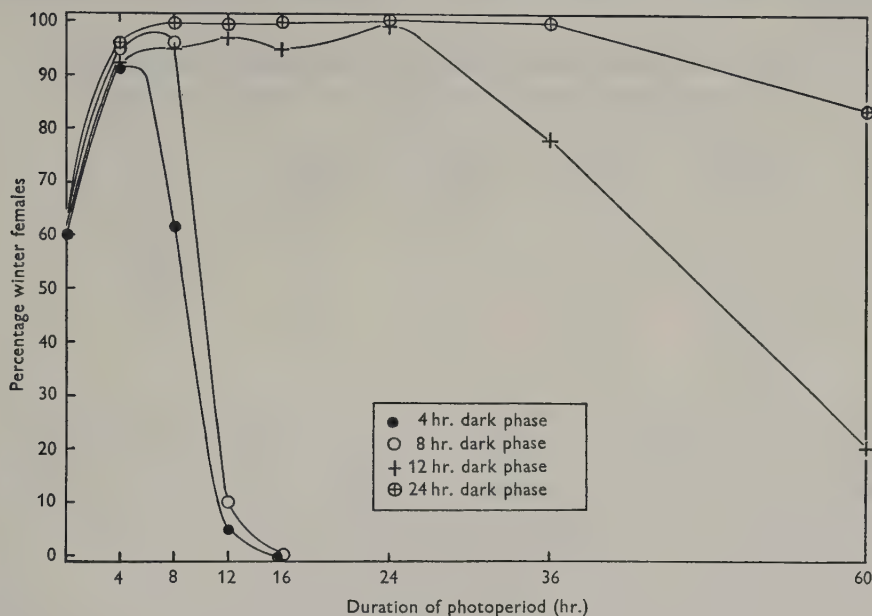


Fig. 1. Influence of the dark phase in the cycle of illumination on the incidence of diapause. Comparison of the 8 and 12 hr. curves shows that the dark phase rapidly becomes more effective in inducing diapause as it is extended to 12 hr.

prevent and the dark phase to induce diapause. However, since the effectiveness of both phases increases most rapidly at a certain critical duration, the existing balance is determined by the absolute length of the phases and not by the ratio of the light to the dark hours in the cycle. A specific instance is provided by the very different results obtained with cycles of 16 hr. light, 8 hr. darkness and 8 hr. light, 4 hr. darkness (Table 1).

INTERRUPTION OF THE DARK PHASE

Much valuable information concerning the light and dark phase reactions which control flowering in many plants, has been gained by interrupting an effective dark period by short light exposures (Borthwick *et al.* 1950). Such experiments have established that flowering is governed essentially by the duration of the dark phase. Flower formation in short-day plants is suppressed, and in long-day plants is initiated, if the long 'night' period is broken by quite short lighted intervals of

30 min. or less. The action of light is largely independent of duration and seems to involve the rapid photosensitized destruction of some product formed gradually in the leaves of the plant during the dark phase.

By comparison *M. ulmi* is highly insensitive to interruption of the dark phase. Table 2 shows the incidence of diapause when photoperiods of increasing duration are introduced into the middle of a long dark period. The time interval from the

TABLE 2. *The influence on diapause of interrupting an effective (diapause-inducing) dark phase by different intervals of light*

Cycle of illumination (hr.)				% winter females
Light	Dark	Light	Dark	
8	16	—	—	100
8	7	2	7	94
8	6	4	6	100
8	5	6	5	76
8	4	8	4	62
8	3	10	3	14
8	2	12	2	0

beginning to the end of the divided dark phase was maintained constant at 16 hr. by reducing the total hours of darkness in the cycle. It may be seen that an interruption of 2 or 4 hr. is without influence on diapause; and even 6 hr. of light only reduced the proportion of winter females to 76%. The effective fusion of the dark periods must indicate that the light phase reaction develops slowly.

These findings are in general agreement with previous observations on *Grapholitha molesta* (Dickson, 1949) and on *Acronycta rumicis* (Danilyevsky & Glinyanaya, 1949). In the first insect the reduction of diapause by as much as one-half requires a 2 hr. light interruption of the long dark phase. In *Acronycta* a 3 hr. light interval introduced into a diapause-inducing 15 hr. dark phase is capable of abolishing diapause completely, but its effectiveness depends on the point of interpolation within the dark phase. Diapause was influenced most strongly when the dark phase was divided equally into two 6 hr. dark phases, since an uninterrupted dark phase of at least 9 hr. is essential for complete diapause. 54% of the pupae still entered diapause when the dark phase was divided into two phases of 3 and 9 hr. and diapause remained almost universal with dark phases of 9 and 3 hr. The authors interpret these differences in terms of the suppressive influence of each phase on its successor but the evidence is insufficient for a full evaluation of the results.

INTERRUPTION OF THE LIGHT PHASE

The light phase reaction in *Metatetranychus ulmi* is almost equally insensitive to interruption by darkness. The effect of the inclusion of dark periods of different duration within a long light phase is shown in Table 3. The 16 hr. photoperiod normally suppresses all diapause. With a 2 hr. dark interval all the mites still

develop as summer females and only 28% of winter females were recorded when the dark interruption was extended to 4 hr. The incidence of diapause reached 89% with an 8 hr. dark interval. In *Grapholitha* only 3 hr. of darkness were required to produce a comparable effect (Dickson, 1949). It seems therefore that the initial development of the dark phase reaction in *Metatetranychus ulmi* must be even slower than in *Grapholitha*.

TABLE 3. *The influence on diapause of interrupting an effective (diapause-preventing) photoperiod by different intervals of darkness*

Cycle of illumination (hr.)				% winter females
Light	Dark	Light	Dark	
8	16	—	—	0
8	7	2	7	0
8	6	4	6	28
8	5	6	5	82
8	4	8	4	89
8	3	10	3	95
8	2	12	2	95

DISCUSSION

The complete curves relating daily photoperiod with the incidence of diapause in *Acronycta rumicis* at 27–28° C. (Danilyevsky, 1948), in *Grapholitha molesta* at 24° C. (Dickson, 1949) and in *Metatetranychus ulmi* at 15° C. (Lees, 1953) have many features in common. Nevertheless, it may not be without significance that these arthropods all have the same requirements, namely to enter diapause in short days and to develop without diapause in long days. A like response to photoperiods experienced as a normal part of the environment (photoperiods, that is, ranging from c. 8 to 17 hr. daily) may therefore be due to adaptive convergence rather than to any fundamental similarity in the mechanism of the photoperiodic reaction.

Although Danilyevsky & Glinyanaya (1949, 1950) assume that the dark phase plays a dominant role in *Acronycta*, it seems probable that all three species react to the duration of both the light and the dark phases. However, the nature of the response to atypical cycles of illumination is entirely dissimilar and indicates that considerable differences in the underlying mechanism must exist. In *Grapholitha molesta* almost all combinations of light and darkness cause uninterrupted growth. Diapause only supervenes if the regime includes a light phase of 7–15 hr. and a dark phase of 11–16 hr. (Dickson, 1949). In *Acronycta* the induction of diapause also requires rather special conditions, namely a dark phase of critical duration. Uninterrupted development takes place when the dark phase is shorter than 9 hr. or longer than 24 hr. Danilyevsky & Glinyanaya (1949, 1950) are of the opinion that unless the appropriate specific light/dark 'rhythms' are engendered, the insect behaves as if continuously illuminated.

The influence of different cycles of illumination on diapause in *Metatetranychus ulmi* is summarized in Fig. 2. It will be noted that the conditions under which diapause may arise are far more extensive than in the two species of Lepidoptera. This is because the course of development is governed by the balance between

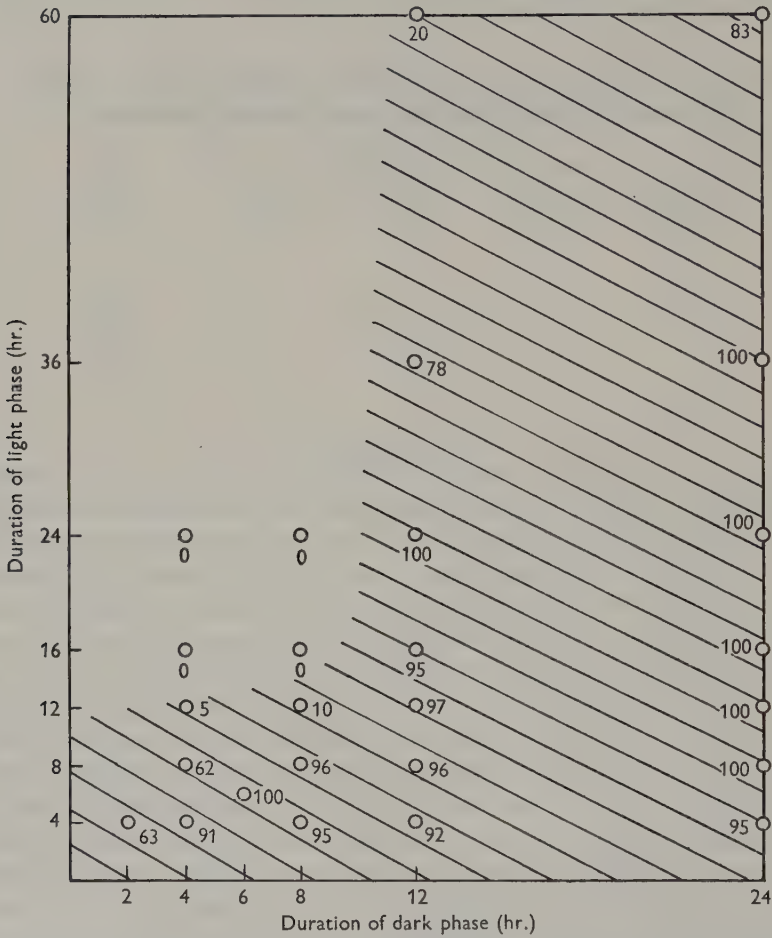


Fig. 2. Chart illustrating the influence of different regimes of light and darkness on diapause in *M. ulmi*. All the combinations which induce diapause are included in the area indicated by hatching. The incidence of diapause in each case is also shown.

diapause-preventing (light phase) and diapause-inducing (dark phase) stimuli and not by the occurrence or otherwise of phases of critical duration. Experiments employing short interruptions of long light and dark phases have shown that both light and dark phase reactions are imperceptible during the initial few hours; but both also gain rapidly in effectiveness when the phases have continued for 8–12 hr.

At the same time, therefore, the response to any specific cycle also depends upon the absolute length of both light and dark components.

Studies concerned exclusively with the role of external factors cannot, unfortunately, provide any sure indication of the mode of action of photoperiod in operating the diapause switch mechanism. Dickson (1949) has suggested that metamorphosis in *Grapholitha* is suppressed by an inhibitory hormone which is under the control of the appropriate light and dark-phase reactions. In *Platysama*, however, Williams (1946) has demonstrated that diapause in the pupa persists until the brain hormone is released by chilling. And Way & Hopkins (1950) point out that the pupal diapause in *Diataraxia* may also be caused by the absence of the brain hormone; long photoperiods, by stimulating the brain through the optic tracts, might then be expected to provoke more neurosecretory activity than short photoperiods and so cause the release of the hormone. If it were found that the duration of the dark phase is also a significant factor in *Diataraxia*, this hypothesis could be broadened by assuming that the brain hormone is dispersed during periods of darkness when nervous activity would be absent.

On the other hand, recent experimental evidence suggests that the diapause condition in the eggs of the silkworm arises from the presence in the blood of the female moth of a 'hibernation' hormone secreted by the suboesophageal ganglion (Hasegawa, 1952). In moths reared in short photoperiods, and therefore destined to lay non-diapause eggs, the brain appears to exert an inhibitory action, through the oesophageal connectives, on the suboesophageal ganglion, with the result that the hormone is withheld (Fukuda, 1951). Diapause, in this instance, is not brought about by the lack of a growth-promoting hormone.

In view of this diversity among comparatively closely related insects, no assumptions as to the internal mechanism of diapause in *Metatetranychus ulmi* can safely be made. However, certain conclusions can be drawn from the systematic character of the response to different combinations of light and darkness. The role of light and darkness in suppressing and promoting diapause respectively suggests that some active substance, capable either of preventing or inducing diapause, is synthesized during one phase and destroyed or otherwise removed from the sphere of action during the complementary phase. Whether synthesis occurs during the light or dark phases cannot be determined from the evidence available. On this basis it seems that (i) neither the rate of synthesis nor removal of active principle is constant. Both increase to a maximum after a certain critical duration (e.g. between 8 and 12 hr. in the case of the dark phase reaction). (ii) Synthesis and removal must be cumulative processes with some residuum of active substance being carried over from one cycle to the next. For example, with winter females 14 cycles of long days and short nights may be without effect whilst 15 are decisive, causing the mite to switch over to summer eggs (Lees, 1953). (iii) The influence which the accumulation or removal of active principle exerts on diapause must also be subject to the

competence of the ovaries to respond. Eight cycles, for example, are sufficient to switch development in the deutonymph, whereas 15 or more are required in the adult.

A simple hypothesis of this kind would account for the prevention of diapause in continuous illumination but not for its partial suppression in the absence of light. The interaction of photoperiod with other factors, e.g. temperature, presents further difficulties. In *Diataraxia oleracea*, Way & Hopkins (1951) have suggested that temperature influences the secretion of the brain hormone directly by controlling nervous activity in the brain. According to this view a diurnally fluctuating temperature should always exert an identical effect upon diapause regardless of whether the higher temperature occurs during the night or day periods.

Experiments by Danilyevsky & Glinyanaya (1950) seem to indicate that *Acronycta rumicis* is sensitive to temperature at least during the light phase. When the larvae were exposed to a 15 hr. (diapause-inducing) dark phase a 3 hr. light interruption only prevented diapause at high temperatures (26° C.). If the interpolated light phase was passed at temperatures of 14° C. or lower, diapause remained universal. In *Metatetranychus ulmi*, however, temperature sensitivity is mainly confined to the dark phase. With a 24 hr. cycle divided into day and night periods of equal length, diapause remained universal if the high temperature (25° C.) was experienced only during the light phase, whereas the incidence of diapause was halved if the period of high temperature coincided with the dark phase (Lees, 1953).

This observation suggests that in *M. ulmi* the action of temperature may be closely integrated with the cycle of illumination. The absence of any temperature effect during the light phase is consistent with the expectation that the thermal coefficient of the photochemical reaction (involving either a synthesis or a photo-sensitized destruction of active principle) would approximate to unity. However, on this basis it is difficult to account for the reduction of diapause with high night temperatures which would be expected to increase the rate of the dark reaction.

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THE EFFECT OF REPEATED SPRAYING OF INSECTS IN INCREASING THEIR RESISTANCE TO INSECTICIDES

I. DEVELOPMENT OF RESISTANCE TO DDT IN A STRAIN OF *DROSOPHILA MELANOGASTER* MEIG.

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(With 8 Text-figures)

Successive spraying with DDT suspensions of the adults of a wild colony of *Drosophila melanogaster* and the progeny of survivors enhanced the resistance to that insecticide.

The rate at which resistance increased depended on: (1) the relative proportion of resistant to susceptible individuals, or (2) the intensity of selection as measured by the concentration of DDT, the proportion killed or on both. The resistance of the populations of the insects fluctuated considerably whether subjected to successive sprayings or not, and in one sprayed series there was some indication of a rhythm with peaks of susceptibility occurring at regular intervals.

Enhanced resistance may show a change of slope in the probit log concentration regression line, leading to different relative values at different levels of mortality, or by a parallel shift of the regression line. The former appears to be a preliminary stage of selection and indicates a change in the frequency distribution within a population.

Increasing the concentration of DDT, slowly or rapidly, may have enhanced resistances at an increased rate, but the series sprayed with the lower initial concentration reached finally the same end point, as judged by the values of log L.C. 50.

During the course of these experiments the insects developed sensitivity to carbon dioxide (used in anaesthesia). Its bearing on our work is considered in Part II.

INTRODUCTION

The development of resistance by insects to the lethal effects of insecticides has been a subject for discussion since Melander (1914) raised the issue. Since then the investigations of Quayle (1916, 1922, 1938) and of Hough (1928, 1929, 1934) and other research workers in America, had, before the 1939-45 war, drawn attention to the fact that the repeated application of certain insecticides could lead to the production of a population of insects which were increasingly difficult to control by the methods previously used with success. Since 1939, and the vastly increased use of insecticides, particularly synthetic organic compounds of a more persistent type, which could be employed as films, permanent over weeks and months, there has been a steady accentuation of the problem of the control of a number of insects and other parasitic arthropods.

A summary of the literature has been issued by Babers (1949) and by Babers & Pratt (1951). Brief reference is made here to that part of the literature dealing with *Drosophila melanogaster*, the insect used in our investigations.

Boyce (1928) subjected his insects to a concentration of hydrocyanic acid. Survivors' eggs were taken, and the progeny, repeatedly exposed to the same treatment, showed a slightly increased resistance. Babers (1949) mentions that Bartlett had been able to select strains from sixteen varieties of *Drosophila* which were resistant to DDT (2:2-bis-(*p*-chlorophenyl)-1:1:1-trichloroethane), hydrocyanic acid and tartar emetic, but the possibility of selection depended on the strain. Bartlett (1952) has subsequently published an account of the susceptibility to several insecticides of a number of strains of *D. melanogaster*. He chose the insect, as did we, because its physical and genetic diversity 'have been subjected to closer scrutiny than any other species of animal'. He found that different strains had different susceptibilities to DDT, tartar emetic and hydrocyanic acid, that resistance was inherited, that successive treatments led to an increased resistance in some strains, but not in others, and that selection for DDT resistance was very slow, a finding in agreement with our own. His work covered other insecticides and he noticed that resistance to DDT was associated with resistance to 2:2-bis-(*p*-fluorophenyl)-1:1:1-trichloroethane (DFDT), but apparently resistance to HCN was, with the most resistant strains, specific and not associated with resistance to DDT or tartar emetic. This strain was less susceptible to diethyl ether and to a subfreezing temperature. Bartlett suggests that selection for DDT resistance is by steps.

Reimschneider & Rohrmann (1950), after prolonged selection through many generations, produced a strain of *Drosophila* more resistant than the original parent strain to DFDT.

An important but not strictly analogous work relates to the ebony strain of *D. melanogaster*, which was found by L'Héritier & Teissier (1937) to be highly susceptible to carbon dioxide, a feature almost unique in this species. L'Héritier (1948, 1949, 1951) has reviewed, very completely, the work carried out by himself and his associates upon this strain. It was of importance to us since shortly following our first experiments in selection spraying, in which carbon dioxide was used for anaesthesia, our colony of *D. melanogaster* developed a marked sensitivity to this gas.

Our work was designed to find if a DDT-resistant strain resulted from repeated spraying with DDT, the progeny of survivors being successively raised, and to ascertain the kind of fluctuation in resistance likely to occur. These fluctuations were magnified by the appearance in our test subjects of CO₂ sensitivity, and later experiments were largely devoted to the analysis of the mutual effects of the factors making for DDT resistance and CO₂ sensitivity, and particularly the effect that the use of carbon dioxide for anaesthesia would have on the separation of DDT-resistant strains by repeated spraying. It was at first considered that a relationship might exist between the two, as the effect of DDT upon insects is to increase greatly

their rate of respiration (Lord, 1949). We were not, however, able to show any major relationship, and it appeared as if the net effect of the administration of carbon dioxide to a CO₂-sensitive strain before spraying was to cut down the population of insects subject to selection. Thus the CO₂ action may have diminished the rate of selection but did not inhibit its final effect.

EXPERIMENTAL

A wild and fairly large colony of *D. melanogaster* was chosen, to approximate to natural conditions and so be more likely to be amenable to selection. However, to acclimatize them to the laboratory and to increase their numbers, they were reared for several generations by the usual laboratory technique, using half-pint milk bottles as containers and an agar-molasses-oatmeal medium inoculated with yeast.*

The insects were sprayed in the adult stage, after anaesthesia with CO₂, used in just sufficient quantity to enable counted lots to be separated, and with a second dose given to maintain their quiescence until they had been sprayed. After the development of CO₂ sensitivity was observed, the extent of exposure was cut down to a minimum and later the room temperature was raised to above 20° C. during spraying. This lowered the CO₂ sensitivity without removing it entirely, and it probably prevented the sprayed stock from being wiped out, during peaks of sensitivity. In later work, nitrogen was substituted for carbon dioxide.

The insects were reared in a constant temperature room illuminated by fluorescent lamps, kept first at 22° C., but as this temperature was too low for adequate reproduction it was raised to 24° C.; humidity fluctuated slightly. The occasional presence of moulds or mites necessitated the discarding of some cultures. Cultures were made by introducing into the bottles a maximum of ten females and ten males from among the survivors of each spraying. A period of 21 days was required at the lower temperature; in order to avoid the appearance of an *F*₂ generation this was reduced, and for some determinations of the L.C. 50, the parents were transferred each week, over a period of 3 weeks, to fresh culture bottles and separate sprayings were carried out on their adult progeny.

The spraying apparatus used was a modification of the Potter tower (Potter, 1941, 1952), but it was much shorter and somewhat wider, thus nearer in its dimensions to the Tattersfield-Morris apparatus (Tattersfield & Morris, 1924; Tattersfield, 1934). It measured: height, 45 cm.; diameter at top, 20 cm.; tapering through a length of 15 cm. to a diameter of 15 cm. with a gap of 10 mm. between

* The medium for the larval cultures was made up to the following recipe; oats, 87 g.; agar, 10 g.; nipagin, 0.74 g.; molasses, 15 ml.; water, 750 ml. The agar was boiled with some of the water and the nipagin added, more water and the molasses added and finally the oats and the remainder of the water. The whole was boiled for half an hour, poured into standard half-pint milk bottles to the approximate depth of 2 cm. When cool, each bottle was inoculated with four drops of a yeast suspension (1 g. yeast to 30 ml. water). A strip of cellulose wadding was enclosed for pupation purposes and the bottle closed by a wad of cotton-wool in a muslin bag.

the tower itself and the adjustable platform. The insect container was much smaller than in the Potter technique as it was deemed advisable to use far less fluid (1 c.c.) in the spray reservoir, and thus to complete spraying before recovery of the insects from anaesthesia. The atomizing nozzle was similar to that used in the Potter tower, but had a needle valve, which enabled the operator to modify the spray to prevent the small flies from being physically damaged. The atomizer was worked by an electrically driven pump, and the pressure of the air stream was adjustable. A rapid rate of application of the spray was, with experience, achieved. The technique of spraying and handling the insects was progressively improved in detail.

The spray apparatus was, for one or two tests, used without a tower in a completely closed cupboard, with the spray nozzle adjusted to give a pressure of 50 cm. Hg, and a deposit on the standard dish (area 10.3 cm.²) of approximately the same amount as was found most satisfactory throughout the experiments (4.9–5.8 mg./cm.²).

After anaesthesia the flies were counted during the early experiments into empty tubes (3.5 cm. diameter, 7.5 cm. high). Just before spraying, a tube was taken and the insects lightly anaesthetized by CO₂ and rapidly transferred to a small dish containing a circle of tricolene. Immediately after spraying they were transferred with the tricolene circle back to the empty tubes, which were plugged with cotton-wool. After about an hour, the flies were transferred to tubes (10 cm. high, 3.5 cm. diameter) containing a layer of agar-molasses medium approximately 1½ cm. deep, and lightly plugged with cotton-wool. They were then kept at 24° C. until inspection.

The early technique had not proved too difficult when spraying a large number of insects with a few concentrations, but, for work demanding greater accuracy, the possible escape of insects recovering from the effects of spraying had to be prevented. Moreover, it became advisable to avoid the effects of starvation for variable periods, and to reduce the time required for the work. Tricolene was also difficult to obtain in sufficient quantity, and circles of hardened filter-paper were substituted. The following modified technique was therefore adopted.

In the bottom of the tubes used for storage of the insects before and after spraying, a layer of cotton-wool was placed, just saturated with molasses solution (approx. 5%) and pressed down. The insects separated from the rearing bottles were counted into the tubes, and transferred as required to the disks of hardened filter-paper (Whatman no. 50), kept in place by a brass ring (3.5 cm. diameter, 1 cm. high) on the upturned bottom of an aluminium dish (3.8 cm. diameter, 1 cm. high). After spraying, the insects were transferred at once to the same storage tubes and kept in the constant temperature room until they were examined (48 hr. later).

After some experience it was decided that, to increase the accuracy of the work and for the determination of regression lines, the insects should, for each concentration, be sprayed alternately, that is, if three stocks *A*, *B* and *C* were used, at

each concentration one tube of *A* would be sprayed, followed by one of *B* and one of *C*, reverting to *A* again for the second tubes and so on. This technique was found to give less heterogeneity in the data and to be expeditious. Speed was an important consideration as the particles of a DDT spray, prepared by diluting a concentrated solution of acetone with a diluted solution of sulphonated lorol, grow in size with time, resulting in a clogged nozzle, a lessened deposit and a change in toxicity. As far as possible the solvent content of the spray was kept at a constant amount for all concentrations of DDT. If acetone had to be added to achieve this, it was first floated on the surface of the measured amount of sulphonated lorol and the DDT solution very gently added, rapidly shaken and sprayed with the greatest expedition, all sprays being timed with a stop-watch.

THE PLAN OF THE EXPERIMENTS

The overall scheme of the experiments is given in the accompanying plan. The process involved the repeated treatments of the adult insects in succeeding generations with dosages of known levels, and breeding from the survivors. The programme involved the determination of the probit-log concentration regression lines, the values for the log median-lethal-concentration m and their slope b . The values for m would give the relative degree of shift in the resistance at the median probit and the slopes b the relative measure of its distribution and the alteration of the relative resistances with change of level in mortality.

In order to decide the concentrations to be used in the series of sprayings, a preliminary log concentration-probit regression line was determined. The data are shown in Table 1.

A highly purified crystalline preparation of DDT was dissolved in acetone (0.4%). Dilutions of 0.02, 0.01, 0.0075 and 0.005% (w/v) were made by diluting with a solution of 0.05% sulphonated lorol. No deaths occurred in the controls sprayed with the carrier.

TABLE 1. *Spraying of DDT-acetone-sulphonated lorol carrier, 2 November 1949*
(Mean deposit 5.75 mg./cm.². CO₂ anaesthesia before spraying. Lab. temp. not recorded.)

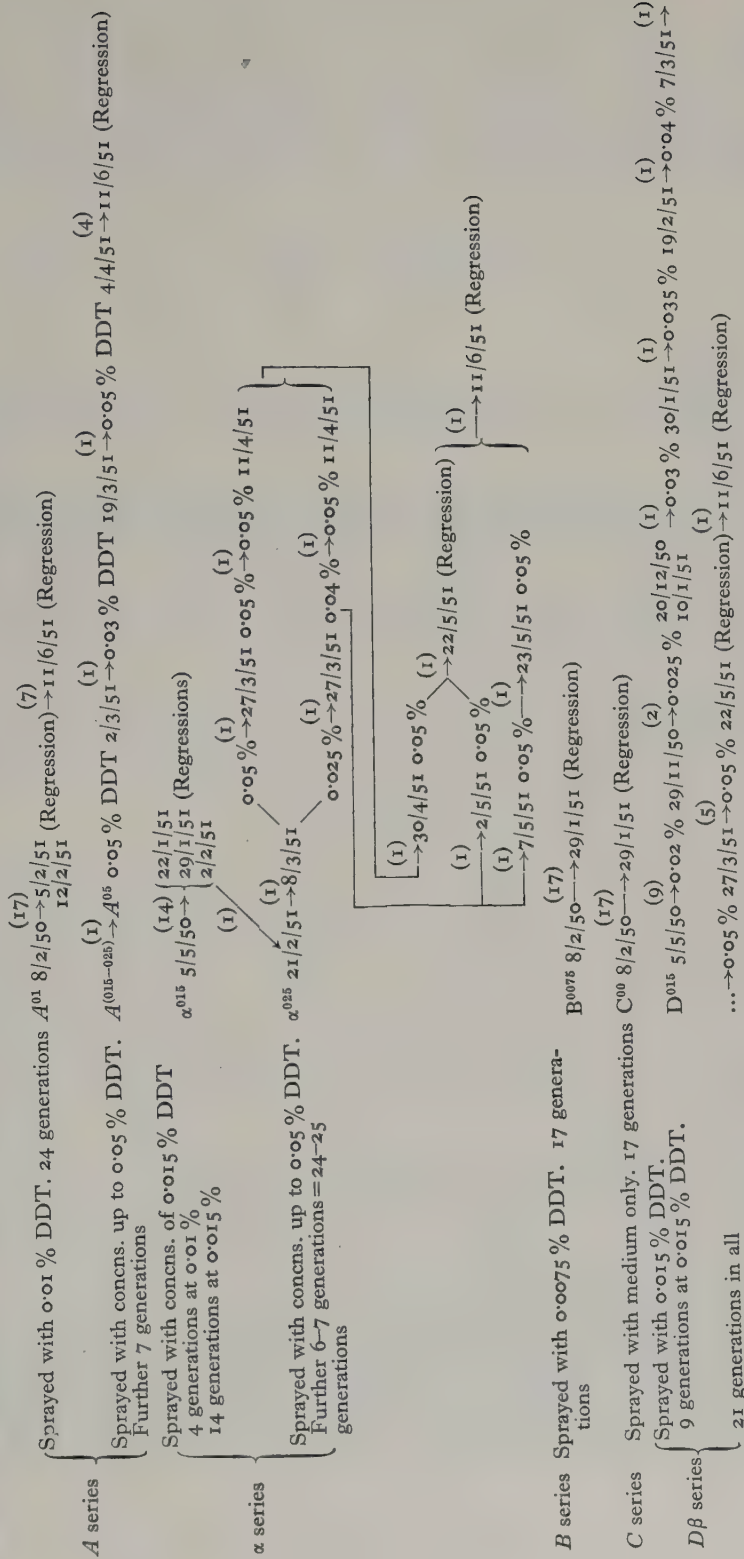
Conc. DDT (mg./1000 c.c.)	Conc. acetone	% dead
200	5.0	90.9
100	2.5	85.7
75	2.5	79.5
50	2.5	44.2
0	5.0	0

$$\chi^2_{[2]} = 5.14, \text{ not heterogeneous. } \log \text{ L.C. } 50 = m = 1.669 \pm 0.069 = 46.7 \text{ mg./l.} \\ b = \text{slope} = 2.5997 \pm 0.5768.$$

In February 1951, a spraying intended to give a preliminary selection was made. A deposit 6.56 mg./cm.² was employed. Concentrations of 100 and 75 mg./1000 c.c.

PLAN OF SPRAYING

The following plan shows the course taken over a period of some 18 months with several separate lines, with the dates and numbers of generations. The nomenclature of the stock (e.g. A) is given with the concentration as the power of the letter. E.g. $A^{0.01}$ = stock A sprayed with 0.01 % DDT. The number of generations is given as a figure in round brackets, (7) = 7 generations. The dates are given as 8/2/50. (Regression) = full scale spraying.



DDT were used, together with a control spraying with the carrier containing 2.5 c.c./100 c.c. pure acetone and 0.05% sulphonated lorol. The available stock of insects for this experiment was 249 females and 204 males. The results were as follows:

	Concn. DDT (mg./1000 c.c.)	Percentage mortality		
		Total	♀♀	♂♂
<i>A</i>	100	93.5	96.6	89.2
<i>B</i>	75	65.8	69.6	60.3
<i>C</i>	0	12.5	16.2	9.2

The insects surviving the treatments *A*, *B* and *C*, were cultured, and throughout this series of tests were labelled *A* (0.01% DDT), *B* (0.0075% DDT) and *C* (carrier only).

After three or four successive sprayings it was clear that with the concentration 0.01% DDT there was an apparent steady decrease in susceptibility to its effects. The small number of test insects used made the significance of this result doubtful, but, since it had taken place, it was decided to continue with successive sprayings to ascertain the degree of resistance to DDT which would develop in this stock, and the effect of raising the concentration and the possibility of selecting a fresh strain from the original stock. Repeated checks were made by spraying the original and hitherto unsprayed stock to ascertain the degree of selection attained.

The dates, numbers of sprayings (generations), the percentages killed and the temperatures are recorded in graphs (Figs. 1-5).

On 21 December 1950 the selected stock of *A* (0.01%) adults was lost during etherization while culturing. Owing, however, to the technique used, a number of eggs had been laid on the medium, and although it was necessary to omit one spraying and to include a number of F_2 flies in the first spraying subsequent to this, the series was continued and eventually (4 April 1951) had reached a point where only about 5% of the flies succumbed to treatment. This was less than the proportion killed in the controls sprayed with the carrier alone. Subsequent sprayings showed variations about this value, but there was no sign of any further decrease in susceptibility. It was decided that this series of experiments could be ended. A final series of tests was made to determine the L.C. 50's to compare the degree of susceptibility of the original unsprayed stock with that of the series sprayed at intervals with 0.01% DDT, and with several series in which the concentration had been raised step by step to a concentration of 0.05% DDT. These results are discussed later (p. 515).

The most noteworthy features in these graphs are the violent fluctuations in the degree of susceptibility shown by the original stock, sprayed once and discarded, whether DDT was used or the carrier only. The fluctuations in susceptibility of the stock *A* (repeatedly sprayed with 0.01% DDT) are also noteworthy, but there is an apparent rhythm with time in the results, which is not so marked in the other

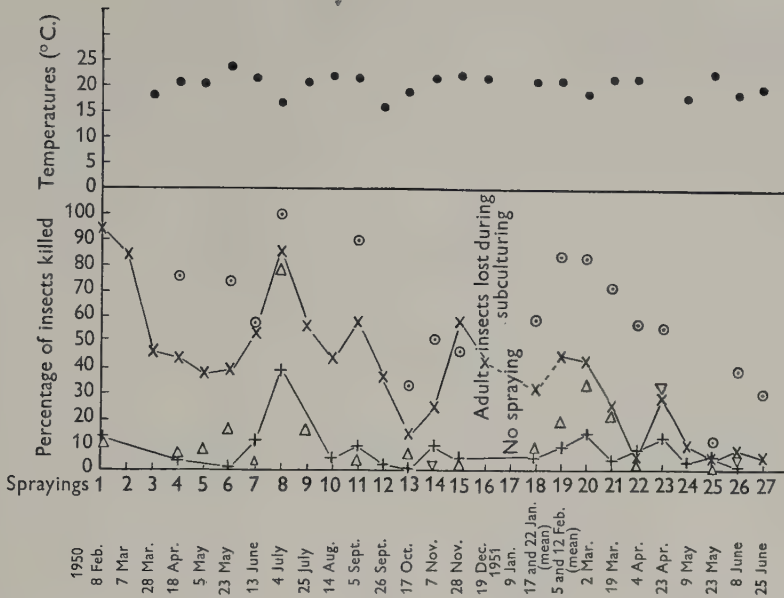


Fig. 1. Percentage of *D. melanogaster* (adults, ♀ and ♂) killed by a concentration of 0.01 % DDT in successive sprayings. *A* stock. Not adjusted to controls. CO₂ anaesthesia before spraying. ×, repeated spraying with 0.01 % DDT (*A* stock); +, sprayed once with medium and discarded (*A* stock); o, original stock sprayed once with 0.01 % DDT and discarded; Δ, original stock sprayed once with medium.

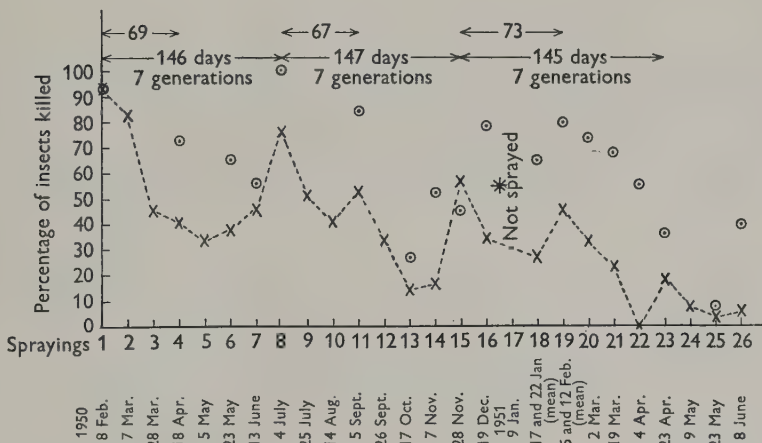


Fig. 2. Percentage of *D. melanogaster* (♀ and ♂) killed by a concentration of 0.01 % DDT in repeated sprayings. Adjusted to controls. *A* stock. CO₂ anaesthesia before spraying. ×, progeny of sprayed insects sprayed with 0.01 % DDT; o, sprayed once with 0.01 % DDT and discarded; *, parents of *A* (sprayed) stock lost while culturing—larvae reared.

series of sprayings. There are major peaks of susceptibility in the *A* series corresponding with sprayings, 1, 8, 15 and 23 (interval of seven generations), and there are minor peaks corresponding with the 11th and 19th sprayings. The major peaks are separated respectively by 146, 147, and 145 days, but the last one is not so strictly defined as the first two, owing to the mishap in culturing which led to an omission of one spraying in order to bring these stocks to a reasonable size. The secondary peaks may be due to chance. There were temperature fluctuations during the sprayings and the first peak is unquestionably partly correlated with the low temperature and high humidity prevailing on 4 July 1950, as all the stocks were affected similarly, but this correlation with low temperature does not apply throughout the series of tests. The incidence of CO₂ susceptibility is probably associated, at least partly, with the effect; it was noticed by the time of the first peak, and is characterized by a negative temperature coefficient, but is sufficiently variable in its incidence as not to be too closely determined in a quantitative sense by temperature. Moreover, when tested for CO₂ susceptibility, the *A* stock, repeatedly sorted out by CO₂ anaesthesia and spraying with DDT, was less susceptible to CO₂ than the parent stock of insects; but so far we have not, by direct experiment, been able to correlate susceptibility to CO₂ with susceptibility to DDT.

The stock subjected to repeated sprayings with DDT reached a low level of susceptibility to DDT in the 13th spray, and despite rather considerable fluctuations, reached a level less than that of a sample of the same insects, sprayed with the medium alone at the 22nd spraying, after which only minor fluctuations took place. With the exception of one very doubtful experiment (24), on one occasion only did the original stock show a greater resistance to the effect of DDT than the one repeatedly sprayed; the parent stock fluctuated also in susceptibility more markedly. Towards the end of our experiments, there was a rapid and consistent decrease in the susceptibility of the parent stock, possibly due to unconscious personal selection and to regularity of culturing. There was, however, no decline in its susceptibility to CO₂ and also, when last compared (13 February 1952), the parent stock still showed a significantly greater susceptibility to DDT than the *A* stock.

After the fourth spraying with 0.01% DDT it was decided to split the *A* stock and use one half for spraying at the higher level of 0.015% DDT (α stock) and at the same time to make a fresh selection from the original stock, using 0.015% DDT for the purpose (*D* β stock). The results are shown in Fig. 3, along with the temperatures of the room at the time of spraying. There may be a very rough inverse correlation of temperature with the percentage kill in the control spraying of the original stock (sprayed once only), but there is no obvious one with the two stocks (α and *D* β) subjected to repeated sprayings. The first peak of susceptibility, so characteristic of the *A* stock sprayed with 0.01% DDT, occurs again in the α series, but a spraying later (9th) with a second peak 7 generations later (148 days) as with the *A* stock. The stock was derived from the *A* stock, and it is only with these two stocks that this characteristic was so markedly observed. It is clear from Fig. 3

that with both α and $D\beta$ stocks, there is, on the whole, a decline in susceptibility with repeated sprayings. It is possible that the more systematic variations shown in the $D\beta$ series may be due to variations in the slope of the log concentration-probit regression line, and that the distribution of resistance within the stock was varying rather rapidly. After the 9th generation (5 May 1950) it was decided to raise

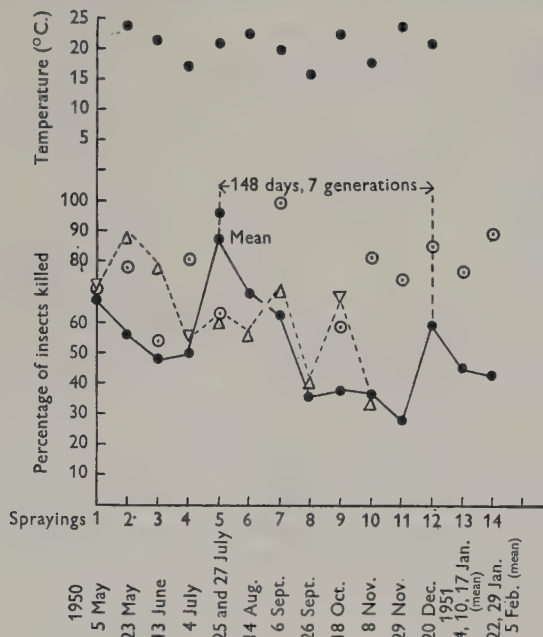


Fig. 3. Percentage of *D. melanogaster* (♀ and ♂) killed by a concentration of 0.015 % DDT in repeated sprayings. Adjusted to controls (α and $D\beta$ stocks). CO₂ anaesthesia before spraying. ●—●, *A* stock progeny of survivors sprayed with 0.015 % DDT from 5 May 1951 (labelled α); ○ ○, original stock sprayed once and discarded; Δ—Δ, original stock progeny of survivors sprayed with 0.015 % DDT, 1st spraying 5 May 1950 labelled $D\beta$.

gradually the level of the concentration sprayed, in the case of the $D\beta$ series. In stages the concentration was raised to 0.05 % DDT and on 19 February 1951 after determining the regression line for the series, a similar procedure was adopted for the α series, the concentration being more rapidly raised to 0.05 %. Similarly, a portion of the *A* series was set aside (2 March 1951) and the concentration sprayed raised to 0.05 % DDT. Beyond the level of 0.04 % DDT a suspension of DDT in an acetone-sulphonated lorol medium could not be used, and a benzene emulsion of DDT was substituted. There was no sign after repeated sprayings that any further selection was being achieved at 0.05 % and the stocks were used for the determination of the regression lines for the different series. The results for these are examined in detail later (p. 515).

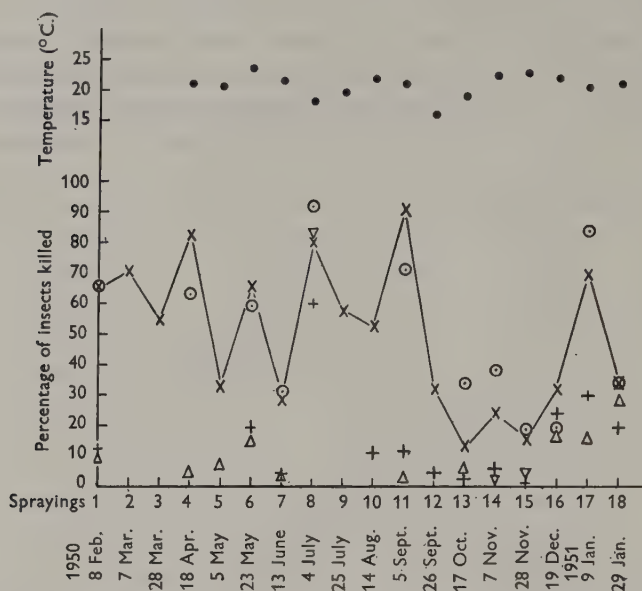


Fig. 4. Percentage of *D. melanogaster* (♀ and ♂) killed by a concentration of 0.0075 % DDT in repeated sprayings. Progeny of survivors sprayed. Not adjusted to controls. B stock. CO₂ anaesthesia before spraying. ×, repeated sprayings at 0.0075 % DDT (B stock); ○, not sprayed (original stock); +, B stock sprayed with medium and discarded; Δ, original stock sprayed with medium and discarded.

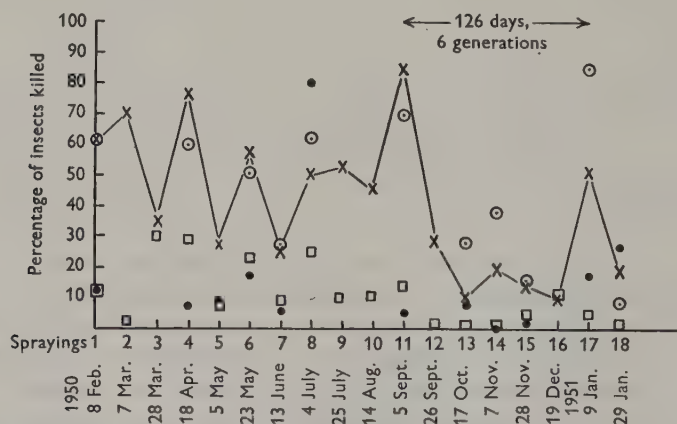


Fig. 5. Percentage of *D. melanogaster* (♀ and ♂) killed by a concentration of 0.0075 % DDT and by the medium in repeated sprayings. Progeny of survivors sprayed. Adjusted to percentage deaths in controls. B stock. CO₂ anaesthesia before spraying. ×, repeated sprayings at 0.0075 % DDT (B stock) control allowed for; ○, not sprayed (original stock) (control allowed for); □, repeated sprayings with medium (C stock); •, original stock sprayed with medium and discarded.

Stocks B and C sprayed with 0.0075% DDT and with the carrier

These series were started on the same day as the *A* series of sprayings (8 February 1950) and the same stock of flies was used. In the *B* series, the percentage of *Drosophila* adults killed at 0.0075% DDT was of the order of 60% against 92% for 0.01% DDT, and selection was much less marked than with the *A* series (0.01% DDT); the apparent fluctuations of susceptibility in the progeny were much more noticeable; indeed the percentage killed at 0.0075% DDT was, on a number of occasions, higher than that shown by the higher concentration of 0.01%. The value of the 8th spray (4 July 1950) was also a very high one, particularly when the control adjustment is not made, but as the controls for both *A* and *B* series were also high, the adjusted values for the number of insects killed by 0.0075% DDT and the degree of their fluctuations are materially reduced (Figs. 4 and 5). The remarks made for the *A* series (p. 506) for this date apply here, and the same excessive sensitivity seen at the 8th spraying was undoubtedly due to a number of factors, which were probably chiefly meteorological.

Probably the most noteworthy feature was the irregularity of the relative results, when those of the *B* series are compared with those obtained with the original stock sprayed once and then discarded. To the very end of the series there was no certainty as to which would show the higher kill. There was, however, no very clear correlation with temperature except with the 8th spray, when a sudden fall of temperature may have accentuated the CO₂ effect, and the high humidity, in preventing evaporation of the medium, may have prolonged, or increased toxic action. The controls, sprayed with the medium only, on this day gave a very high kill, 80% with the original stock and 60% with the stock which had been successively sprayed with 0.0075% DDT. The regression line for this series was determined after the seventeenth treatment. The results are examined later (p. 514).

The series labelled *C*, derived from the same wild colony used in the *A* and *B* series, was sprayed with the carrier only. The progeny of survivors were used for the following test. The medium consisted of 2.5% acetone (w/v) and 0.05% (w/v) of sulphonated lorol (double strength). The percentage kill fluctuated in a way very similar to that of the *B* series but was lower. Until very near the end of the experiment, the results as expressed in probit mortalities lie close to those obtained with the original stock. There are, however, exceptions, particularly after the 15th spray. The log L.C. 50 was determined along with that of the *B* series, and a comparison made with that of the original wild stock (p. 514).

It would appear from these two series that very little increase occurred in the resistance of the stock due to spraying with 0.0075% DDT or with the carrier. It is certainly not much more in either series than the increase taking place in the original stock in the later stages of these experiments.

THE DETERMINATION OF THE REGRESSION LINES AND LOG L.C. 50

That there is a positive change in the susceptibility of the different cultures specified above, and its quantitative degree, can only be ascertained with certainty from the regression lines, and the determination therefrom of the log L.C. 50's and their slopes. The log concentration-probit regression lines for the several cultures were determined. Some experience in an enlarged and more elaborate technique had to be gained before this could be achieved. At first alternate spraying of a number of replicates of one culture and one concentration followed by those of a second culture at the same concentration was adopted, but heterogeneity, sometimes of a high order, appeared in the data, and finally it was decided to adopt the technique outlined on p. 501. In addition, during the α_3 series the substitution of hardened filter-paper for tricolene was made (p. 501).

*L.C. 50 of stock (α) repeatedly sprayed with 0.015% DDT,
and of the original stock*

The α stock was derived from the *A* stock (p. 506). After the fourth spraying the concentration was raised from 0.01 to 0.015% DDT and the progeny of survivors sprayed at regular intervals with that concentration. The data are shown in Fig. 3. It was clear that from the 10th to 12th spray there was a lower susceptibility in this stock than in the original stock. It was decided to carry out tests on the same generation at weekly intervals at a concentration of 0.015% DDT for a period of 3 weeks. For this purpose the parents were transferred to fresh culture media each week, and their adult progeny sprayed after a fortnight in each case. The results were:

Series	Percentage mortality		
	1st transfer	2nd transfer	3rd transfer
	(α_1) 36.03	(α_2) 73	(α_3) 24.8
Original stock	(O_1) 72.7	(O_2) 96.7	(O_3) 59.7

The number of insects was greater in the second culture and there may have been some overcrowding. The survivors were recultured and the log concentration-probit regression line determined. The second culture proved unsatisfactory, and there was much heterogeneity in the data, and it was only after discarding two points of very little weight, the tests for which were carried out at a low temperature at the start of the experiment, that this could be eliminated. The only use made of these data was to incorporate the three points from α_2 and the five from the O_2 series in the regression lines of the combined data in order to illustrate the profound effect of change of slope upon the relative susceptibilities at different levels of mortality. The data are given in Table 2, and Fig. 6, which show that the regression lines for the successively sprayed insects have not only greater values for m than the corresponding original stocks, the differences being significant in the first and last

series of spraying (α_1 and α_3), but that the slope of the regression lines in these series is less than that for the original stock. This effect may be the initial stage in the selecting of a stock showing greater resistance to the effect of an insecticide. The percentage of insects killed increases more rapidly with concentration in the unsprayed than in the sprayed series, so that the value for the log L.C. 50 for the

TABLE 2. *Resistance to DDT of Drosophila melanogaster repeatedly sprayed with 0.015% DDT (α series) and unsprayed (O series)*

(CO₂ anaesthesia before spraying. Spraying dates; α_1 and O₁, 22 Jan. 1951; α_2 and O₂, 29 Jan. 1951; α_3 and O₃, 5 Jan. 1951.)

Series	log L.C. 50	Slope (<i>b</i>)	χ^2
	(mg./1000 c.c. DDT)		
α_1	2.183 \pm 0.043	3.34 \pm 0.88	8.98* (3 D.F.)
α_2	2.274 \pm 0.186	1.84 \pm 1.30	2.30 (1 D.F.)
α_3	2.321 \pm 0.022	3.92 \pm 0.55	1.47 (3 D.F.)
O ₁	1.922 \pm 0.047	5.25 \pm 1.22	16.19** (3 D.F.)
O ₂	1.979 \pm 0.023	6.15 \pm 0.82	9.53* (3 D.F.)
O ₃	1.904 \pm 0.025	5.21 \pm 0.61	1.41 (3 D.F.)
Combined α 's	2.245	2.70 \pm 0.27	42.63** (11 D.F.)
Combined O's	1.944 \pm 0.018	5.61 \pm 0.53	37.91** (13 D.F.)

* $P < 0.05$. ** $P < 0.01$.

Analyses of χ^2

α series	D.F.	χ^2	M.S.
Differences between slopes	2	4.98	
Differences between <i>m</i> 's (common slope)	2	24.90	
Residual	7	12.75	
Total	11	42.63	
O series			
Differences between slopes	2	2.02	
Differences between <i>m</i> 's (common slope)	2	8.76	4.38
Residual	9	27.13	3.01
Total	13	37.91	2.92

In computing the standard errors, the following heterogeneity factors have been used:

α_1 and O ₁	4.20 (6 D.F.)
α_2 and O ₂	2.96 (4 D.F.)
Combined O's	2.92 (13 D.F.)

The values of *m* for the α series differ significantly, and no standard error is therefore given for the log L.C. 50 of the combined line. The three slopes are not significantly different. The O lines do not differ significantly in either slope or position, having regard to the overall heterogeneity of the points.

α series occurs at 2.2451 and the original stock at 1.9442; but the log L.C. 95 for the α series occurs at 2.8536 and the original stock at 2.2373, i.e. that compared at the L.C. 50 the sprayed stock is about twice as resistant as the original stock, and at the L.C. 95 some four times. In the α_3 series in which no heterogeneity in the data was found, the log L.C. 50 (*m*) is 2.3214, and with the original stock 1.9036. Log L.C. 95 is 2.7512, and with the original stock 2.2196. Thus in this case, at the

L.C. 50 the original stock is 2.6 times as susceptible as the sprayed stock, and at the L.C. 95 approximately 3.4 times.

The frequency curve (Fig. 6*b*) indicates a relatively large reduction in the least and medium resistant strains in the repeatedly sprayed stock, and an increase in the more resistant strain or strains. The curve is obviously very skew, and it is

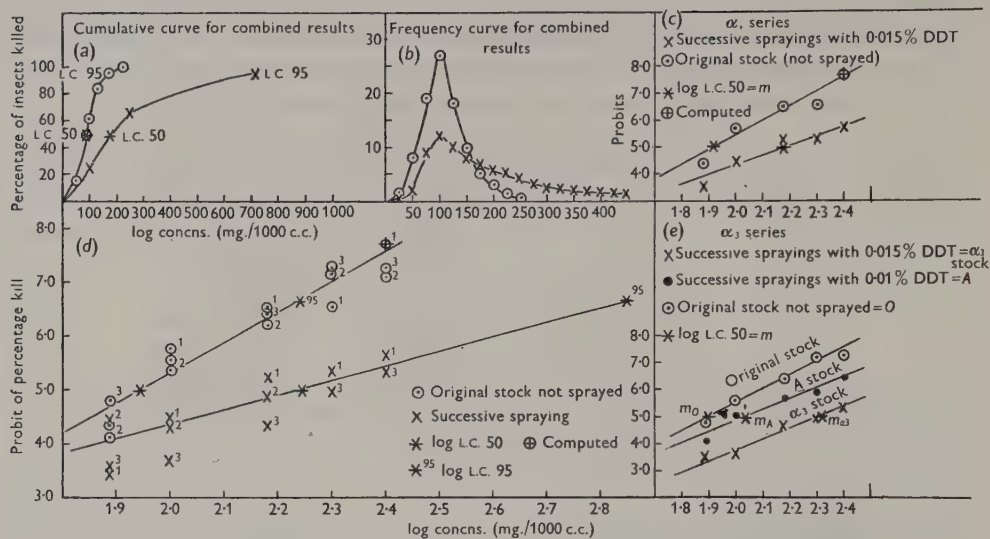


Fig. 6. The effect of successive sprayings of *D. melanogaster* (φ and δ) with 0.015% DDT. CO_2 anaesthesia before spraying. α series. Deaths in controls allowed for.

doubtful whether a slope resistance difference of this type would be permanent. In the case of the α_3 series which showed no heterogeneity in the data for either the repeatedly sprayed stock or the original stock, although the departure from parallelism of the regression lines does not quite reach significance the value for the slope is nevertheless smaller with the α series sprayed at 0.015% than it is with the original stock.

The regression line for a sample of the *A* stock repeatedly sprayed with 0.01% DDT for some 11 months, tested at the same time, shows a similar slope to that of the α series but a distinctly lower value for m , and in spite of the evident fluctuations in susceptibility of the various stocks, it is not unreasonable to consider that selection has proceeded more rapidly in the α stock than in the *A* stock from which it was derived.

The determination of the L.C. 50's of the stock sprayed with 0.01% DDT A series

The sets of the stock *A* (A_1 and A_2) were built up by transferring the parents after a week to fresh bottles. The adult progeny of the latter stock (A_2) were sprayed 1 week after the first (A_1). A third series (A_3) was set up, but an attack of mites prevented its use.

The summarized data are set out in Table 3, together with an analytical comparison of the results. No heterogeneity was found in the data as determined in the individual series, but as is seen from the table, when combined, the data were heterogeneous. Both the individual series and the combined results are shown in the table and emphasize again the extent to which fluctuations can occur in closely parallel determinations. The differences in the values for m , the log median lethal concentrations for the series A_1 and O_1 are significant as they are for A_2 and O_2 ,

TABLE 3. *Resistance to DDT of Drosophila melanogaster repeatedly sprayed with 0.01 % DDT (A series) and unsprayed (O series)*

(CO₂ anaesthesia before spraying. Spraying dates; A_1 and O_1 , 5 Feb. 1951; A_2 and O_2 , 12 Feb. 1951.)

Series	log L.C. 50	Slope (b)	χ^2
	(mg./1000 c.c. DDT) (m)		
A_1	2.032 ± 0.023	3.96 ± 0.46	4.91 (3 D.F.)
A_2	2.180 ± 0.021	2.96 ± 0.35	3.37 (3 D.F.)
O_1	1.904 ± 0.025	5.21 ± 0.61	1.41 (3 D.F.)
O_2	1.734 ± 0.074	3.27 ± 0.73	1.03 (2 D.F.)

A slightly aberrant point at a dose of 200 mg. has been omitted from the O_2 line. The slopes of A_1 and O_1 do not differ significantly, neither do those of A_2 and O_2 , but the slopes are markedly less on the second date of testing.

In view of the significant differences in slope and position (reflected in the high values of χ^2) no standard errors are given for the combined lines.

Combined A 's	2.110	3.204	42.92 (8 D.F.)
Combined O 's	1.825	3.906	27.16 (7 D.F.)

The relative sensitivity of A_1 and O_1 is 1.34; that of A_2 and O_2 is 2.79, the value of the relative sensitivity found in the combined line is 1.93.

A_1 being 1.3 times as resistant as O_1 , and A_2 2.8 times as resistant as O_2 . The combined data give a value for the A series of 1.93 times that of the O series for the relative resistance measured at the median lethal concentration.

The change in relative resistance between the two series is due to the fact that there has been a significant increase in the susceptibility of the insects in O_2 as contrasted with O_1 , that is, the proportion of the more susceptible has increased in the O series, whereas in the sprayed stock the proportion has decreased in the A_2 as contrasted with A_1 . This result is in keeping with the rather violent fluctuations shown for the values obtained during our 'selection' sprayings at one concentration, and it is clear that at this stage, selections by spraying with both 0.015 and 0.01 % DDT are not complete. On the basis of these results and to accelerate results it was decided to raise the concentrations used in 'selection' spraying while maintaining a part of one series (A) at 0.01 % DDT. The α stock, and the $D\beta$ stock and a portion of the A stock were therefore used in a set of experiments in which the concentrations of DDT used were raised to 0.05 % (p. 515). The results are given on p. 516.

*Determination of the log L.C. 50's. Series B (0.0075% DDT)
and C (medium only)*

The relevant data, including two control series, one sprayed before the tests with *B* and *C* and the other carried out alternately with them, are given in Table 4. and Fig. 7. An analysis of χ^2 indicates that the regression lines cannot be regarded as differing from parallelism, and the data do not show heterogeneity.

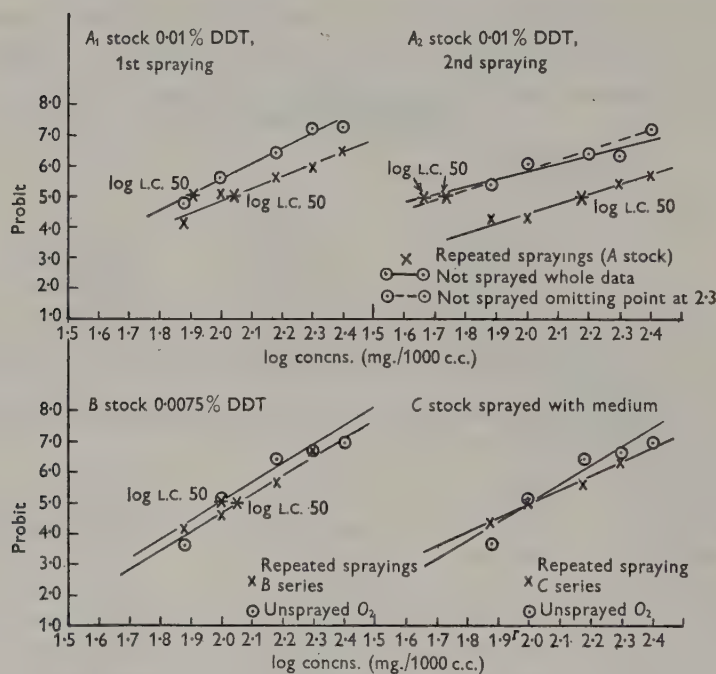


Fig. 7. The effect of repeated sprays of *D. melanogaster* (♀ and ♂) with 0.01 and 0.0075% DDT and with medium. CO₂ anaesthesia before spraying. Deaths in controls allowed for.

The figures presented in Table 4 and Fig. 7 show that the degree of selection in these series is not so marked as with the *A* series (sprayed with 0.01% DDT). Indeed in the *B* series it is only just detectable and the insects repeatedly sprayed with the medium (*C* series) show no signs of being more resistant than the unsprayed original stock at the time of these tests. Whether in the *B* stock this is due to an initially low kill, 60% (as against 92% in the *A* stock) having only a relatively low selective power or whether there was an absence of the more resistant strain in the initial population in this case, it is difficult to decide. The latter view is rendered rather unlikely since there has been, over the period, a slight movement in the *B* stock towards a lower susceptibility, and the fluctuations over the whole

TABLE 4. *Resistance to DDT of Drosophila melanogaster repeatedly sprayed with 0.0075% DDT (B series), with medium (C series) and unsprayed (O series)*

(CO ₂ anaesthesia before spraying.)			
Series	log L.C. 50 (<i>m</i>)	Slope (<i>b</i>)	χ^2 (3 D.F.)
<i>B</i>	2.052 ± 0.019	5.90 ± 0.67	2.03
<i>C</i>	2.011 ± 0.021	4.63 ± 0.55	1.95
<i>O</i> ₁	1.964 ± 0.018	5.83 ± 0.55	6.65
<i>O</i> ₂	2.005 ± 0.019	6.29 ± 0.74	5.44
Combined <i>O</i> 's	1.984 ± 0.018	5.99 ± 0.44	14.19 (8 D.F.)
Analysis of χ^2			
		D.F.	χ^2
Differences between slopes		3	4.33
Residual		12	16.07
Total		15	20.40

The four lines do not differ significantly in their slopes. *B* is slightly more resistant than the combined *O*'s, the relative sensitivity being 1.17. *C* does not differ significantly from the combined *O*'s.

period are so marked as to indicate that it is anything but homogeneous. It is noteworthy that for several sprayings after the second one the percentage mortality in the *A* stock was lower than with the *B* stock.

The effect of raising the concentration

Part of the *A* stock was subjected to a higher concentration, 0.015% DDT, on 5 May 1950, and labelled α stock. The two series were continued side by side, the *A* stock at 0.01% and the α at 0.015%, until the determination of the L.C. 50. On 5 May 1950 a number of the original wild stock were also sprayed and the progeny of this stock periodically treated with 0.015% DDT. On 29 November 1951 this stock (*D* β) was sprayed with 0.02% DDT and afterwards as shown later (p. 517). The concentrations used in the α series were raised to 0.025% on 19 February 1951 and then by rapid stages to 0.05%, a benzene emulsion being used on 8 March 1951 and periodical spraying continued with it until 11 June 1951. The *A* stock was sprayed on 2 March 1951 with an acetone-sulphonated lorol suspension of DDT of 0.05% concentration, but of 600 insects only twenty-four survived. There were no survivors in the original unsprayed stock. The concentration was reduced to 0.03% in benzene emulsion giving 36.36% mortality against 88.77% in the case of the original wild stock. It was subsequently again raised to 0.05% and continued at this level until 11 June 1951 without any reduction of mortality below 90%. The determination of the L.C. 50 was made on that date of the five stocks *A*⁰¹ (repeated sprayings at 0.01% DDT), *A*⁰⁵ (the same stock sprayed with 0.05% DDT), α ⁰⁵ (the α stock sprayed with 0.05% DDT), *D* β ⁰⁵ (*D* β stock sprayed with 0.05% DDT) and the original wild stock *O*. The data are set out in Table 5 and Fig. 8.

TABLE 5. *Resistance to DDT of stocks of Drosophila melanogaster sprayed with different concentrations of DDT*(CO₂ anaesthesia before spraying. Spraying date: 11 June 1951.)Series A^{01} repeatedly sprayed with 0.01 % DDT. A^{05} derived from stock, spraying level increased by rapid steps to 0.05 % DDT. α^{05} derived from α stock (sprayed at 0.015 % DDT), spraying level increased by steps to 0.05 % DDT. $D\beta^{05}$ derived from original stock by repeated sprayings at 0.015 % DDT, spraying level subsequently raised by steps to 0.05 % DDT.

O original stock not previously sprayed.

Series	log L.C. 50 (<i>m</i>)	Slope (<i>b</i>)	χ^2
A^{01}	2.483 ± 0.021	3.84 ± 0.50	2.72 (3 D.F.)
A^{05}	2.495 ± 0.021	4.51 ± 0.48	3.43 (2 D.F.)
α^{05}	2.482 ± 0.019	4.89 ± 0.51	2.39 (3 D.F.)
$D\beta^{05}$	2.351 ± 0.019	4.29 ± 0.33	2.77 (3 D.F.)
O	2.155 ± 0.023	3.70 ± 0.27	2.12 (3 D.F.)
Combined A^{01} , A^{05} and α^{05}	2.486 ± 0.011	4.41 ± 0.29	10.63 (12 D.F.)

Analysis of χ^2	D.F.	χ^2
Differences between slopes	4	5.88
Residual	12	13.43
Total	18	19.31

The five lines do not depart significantly from parallelism. The relative resistances of the combined A 's and α , the $D\beta$'s and the O's are 2.14:1.57:1, the differences being significant.

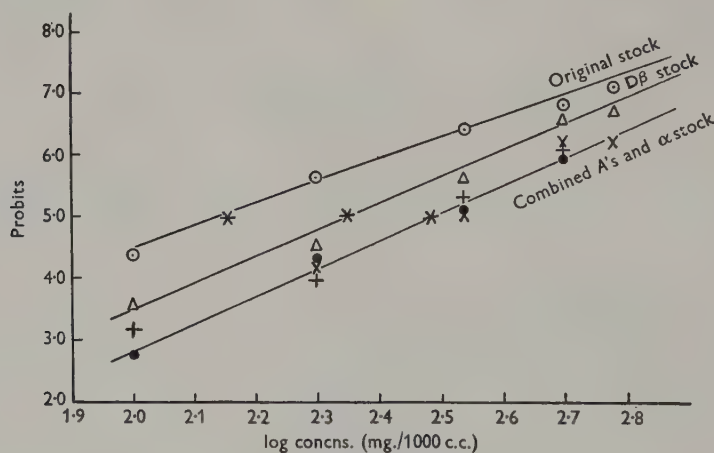


Fig. 8. The effect of raising the concentration of DDT on its selection for susceptibility. \odot , original stock, not sprayed; \bullet , stock A^{01} , repeated sprayings at 0.01 % DDT (A stock); \times , stock A^{05} , repeated sprayings at 0.01 % and 0.05 % DDT; $+$, stock α^{05} , repeated sprayings at 0.01, 0.015 and up to 0.05 % DDT; Δ , stock $D\beta^{05}$, repeated sprayings at 0.015 and up to 0.05 % DDT; $*$, median lethal concentration.

It is clear that one regression line will represent without heterogeneity the results for the series A^{05} , α^{05} , A^{01} . Thus, raising the concentration from 0.01 to 0.05% DDT either in slow or rapid stages, may have accelerated the rate of the increase in resistance, but the lower concentration of 0.01% has achieved the same end-point by the date these tests were carried out. There is a relatively large and significant difference between the value for m (log median lethal concentration) for the series and that for the original stock. The difference indicates that the DDT-selected stocks are of the order of 2 to 2.25 times as resistant to the effects of DDT as the original stock, and this despite the fact that the original stock was markedly less susceptible than that at the beginning of the experiments (29% being killed by 0.01% DDT as against 85–95%).

The regression line for the stock labelled $D\beta$ was determined at the same time as those for the A and α series; it lies between the line representing the combined data for these series and that of the original stock. The $D\beta$ stock was a new selection made from the original stock, but at a later date, using a concentration of 0.015% DDT at the time the α series (also at 0.015%) was derived from the A stock (5 May 1950). The initial mortality was 71%. Spraying was continued at 0.015% through 9 generations (Fig. 3 and Plan) until 29 November 1950, when the concentration of DDT used was raised to 0.02% which gave a kill of 31.9% against 79% with the original stock; the concentration of the spray was raised step by step through 6 generations till it reached 0.05% and continued at that level for a further 5 or 6 generations when regression lines were determined, along with those of the α stock derived from the A stock sprayed for 4 generations at 0.01% and then for 14 at 0.015%. An attempt was made to raise the concentration with which the α stock was sprayed through rapid stages to 0.05%, but this proved difficult and to avoid the stock's extinction it had, before the determinations of the regression lines on 22 May 1951 and 11 June 1951, to be crossed with the α stock raised by slower stages to 0.05% DDT (Plan, p. 503). The data for the two comparisons of α and $D\beta$ are shown in Table 6, which shows that the value for m in the α series has risen significantly between the two dates, but with the $D\beta$ stock the value has fallen but *not* significantly. The mean m 's for α and $D\beta$, 2.40 and 2.37 respectively, are not significantly different but the slopes for both series fall between the two dates. The results suggest that neither stock is quite homogeneous, but that the $D\beta$ series, a new line of selection from the original stock, is approximating to the same stage of resistance as the α series.

The main conclusion to be drawn from these results is that selection for resistance to DDT can be made from these insects by successive sprayings, that the higher the initial mortality, the more rapid the selection. Increasing the concentration when this preliminary selection has been made, may increase the rate of development in resistance, but a strain of comparatively the same order of susceptibility may result from the use of the lower concentration, if the latter is persistently applied for a long enough period. Thus selection would seem to depend on the

distribution of resistance in the original population, and there is clear indication of a lack of adaptation in this insect to an increased potency of an insecticide as measured by the concentration.

TABLE 6. *Comparison of two stocks of Drosophila melanogaster sprayed with 0.015% DDT, the spraying level being subsequently raised to 0.05% DDT*

			Number of generations at		
			0.015% DDT	Increasing levels	0.05% DDT
α_1 crossed with α^{05}			{ 14	2	4
			{ 14	4	1
α^2 crossed with α^{05}			{ 14	2	4
			{ 14	4	2
$D\beta_1$			9	6	5
$D\beta_2$			9	6	6

Series	Date (1951)	Temp. (° C.)	log L.C. 50 (m)	Slope (b)	χ^2
α_1	22 May	20	2.326 ± 0.023	5.28 ± 0.32	0.63 (2 D.F.)
α_2	11 June	19	2.482 ± 0.019	4.89 ± 0.26	2.39 (3 D.F.)
$D\beta_1$	22 May	20	2.390 ± 0.018	4.66 ± 0.15	2.60 (3 D.F.)
$D\beta_2$	11 June	19	2.351 ± 0.019	4.29 ± 0.11	2.77 (3 D.F.)

The difference $m\alpha - mD\beta$ for the first date is -0.064 ± 0.029 ; for the second date it is $+0.131 \pm 0.027$. The slopes of the two lines do not differ significantly on either date.

THE EFFECT OF SELECTION ON RESPIRATION

The rates of respiration of the DDT-selected and the original stock were compared, Barcroft respirometers as modified by Dixon (1951) being used. The temperature of the tests was 25° C. The males and females were separated and transferred to small tubes covered with nylon to prevent their escape into the respirometer flasks. A small drop of honey was placed on the nylon at the top of the tube.

Readings were taken of the volume changes at intervals of 10 and 20 min., and the mean rates of respiration determined in ml./10⁶ per insect per minute, and per body weight. The mean weight of the females was taken as 1.31 mg. and the males as 0.82 mg. The data are set out in Table 7.

TABLE 7. *Respiration rates of Drosophila melanogaster in a DDT-resistant strain and in the stock from which it was derived*

(Mean rate/insect—expressed as ml./10⁶ per min. at N.T.P. No. of tests in brackets.)

	Female	Male
<i>A</i> (resistant)	68.5 (5)	40.9 (6)
<i>O</i> (original stock)	56.1 (6)	42.1 (4)
Differences, <i>A-O</i>	+12.4	-1.2
S.E. of differences	± 4.8	± 7.3

The differences in rates of respiration between the two series are given. It was apparent that body size had a bearing upon rate of respiration, the males being smaller than the females, but a complicating factor is the degree of activity shown by the two sexes. Excision of the wings appears to reduce the rate of respiration in the males more than in the females, but more investigation is needed in order to ascertain whether this technique can be relied upon to have no other effect than reducing random movement. It was not employed, therefore, in this series of experiments.

The results show that the rate of respiration with the females is just significantly higher in the DDT-resistant stock *A* than in the original stock, whereas they are of the same order in the males.

Fullmer & Hoskins (1951) found in female houseflies that resistant strains and susceptible strains had respiration rates of the same order. In our results the differences in rate of respiration for resistant flies is only just significantly larger than with the susceptible flies. Fullmer & Hoskins found the application of DDT to resistant flies induced a smaller rise in the rate of respiration than it did in the DDT-susceptible flies.

Mr B. J. Harrison, of the John Innes Horticultural Institution, kindly examined the *A* stock of *Drosophila* and observed a higher average number of spermathecae in this selected stock. There would not seem to be any direct connexion between the observation and DDT resistance, but it may have some physiological relationship with the length of the life cycle. Pimentel, Dewey & Schwardt (1951) have noted a longer larval period in DDT-resistant houseflies as compared with non-resistant ones.

DISCUSSION AND CONCLUSIONS

A series of experiments was carried out in which the adults of a recently cultured wild colony of *Drosophila melanogaster* were repeatedly sprayed with a suspension of DDT and in the last stages at higher concentrations with an emulsion of DDT, the F_1 progeny of survivors being reared for this purpose. It was found that when, in the initial stages, a mortality ensued of 93% with a concentration of 0.01% DDT, there was a fairly rapid initial selection of a less susceptible insect population. A lower kill of 61% which ensued upon the use of 0.0075% DDT did not lead to any marked selection, and in the course of a few further sprayings a lower mortality indeed was observed in the stock selected with the higher concentration, when sprayed with the higher concentration. Fluctuations in susceptibility sometimes of a large order ensued in the stocks sprayed by 0.01, 0.0075% DDT, and with the carrier alone, as well as with the unsprayed original stock, and these were probably accentuated by an increasing susceptibility to CO₂ which was used for anaesthesia before spraying. In the insects sprayed with 0.01% DDT and later with those sprayed with 0.015% DDT there appeared to be a certain long period rhythm, the peaks of susceptibility occurring at intervals of 7 generations and sprayings

(146–148 days). After successive sprayings for 16 months the stocks treated with 0.01 % showed no lethal effect. Determinations were made of the log L.C. 50's: (i) of this stock after a lapse of approximately 1 year, (ii) of the stock repeatedly sprayed with 0.0075 % DDT, and with the carrier, and (iii) of a stock in which the concentration had been raised from 0.01 to 0.015 % DDT. It would appear from an examination of the log concentration-probit regression lines that the effect of repeated spraying is twofold, giving rise to (a) a change in slope, and (b) a shift in the value of m (median lethal concentration). The change in slope may be only temporary, and is probably the initial effect of spraying due to a change in the distribution of resistance in a stock. The line for the sprayed stock may have a smaller angle of slope, and this leads to a higher comparative resistance at the higher range of mortality. The data indicated, however, that fluctuations in this respect may be considerable in both successively sprayed and unsprayed stocks. The shift in position in the regression line of the successively sprayed stock is probably a more permanent one, and the rate at which it takes place may depend upon (1) the composition of the original population, (2) the concentration of the insecticide used for selection, the higher concentrations giving a higher mortality and therefore a more intense selection. For example, after 1 year from the start, the degree of selection was found to be in the order 0.015 % > 0.01 % > 0.0075 % > carrier alone.

After the treatments were continued for another period of 5 months at 0.01 % and at progressively higher concentrations, further determinations of the probit log concentration regression lines were made. These showed that the stock selected by spraying with 0.01 % DDT was now possessed of a resistance to DDT of the same order as those other stocks derived from it, but which had been treated successively for a number of generations with higher concentrations. All the data for them could, without heterogeneity, be represented by a common straight line. Thus the stock sprayed with 0.01 % had 'caught up', and no further spraying with higher concentrations has so far produced any evidence of further increases in resistance. The evidence is not in favour of there being any adaptation to the higher concentrations of DDT, and although there is some evidence that raising the concentration may accelerate selection for higher resistance, the end-point achieved depends apparently upon the content of resistant strains in the initial population.

Towards the end of the series of experiments outlined above, there was, with large fluctuations a rapid decline in the susceptibility to DDT of the stock originally sprayed. It is not easy to suggest a cause; however, the regularity of subculturing and the care taken to avoid overcrowding may have had a distinct bearing on this. Every care was taken to avoid contamination of cultures with DDT. Nevertheless, there appears to have been a redistribution of resistance levels and the stock at the end showed definite indications of increasing difficulty for selection purposes, but the original untreated stock when compared side by side with the *A* (selected) stock proved in our tests always more susceptible to DDT.

The degree of resistance in our selected stock relative to the original stock is *not* however, a constant, owing to fluctuations in the susceptibility of both, some of which may not be of a genetical character. In our experiments with *Drosophila* the range of resistance was increased by successive sprayings and breeding from the survivors by approximately 1.5 to 3 times, and this superiority endured for many months without further selective treatment with DDT.

When these experiments were well under way, a marked susceptibility to CO₂ developed and proved a complicating factor in analysing our results. A series of experiments was therefore carried out in an attempt to ascertain what part, if any, CO₂, used for anaesthetic purposes, would have in the selection for DDT resistance. The experiments are described in the second part of this series of papers.

We wish to express our indebtedness to Mr R. W. Kerr for much help in developing techniques used during the course of this work and to Dr C. Potter for valuable criticism and advice. We wish to express our thanks to Mr B. J. Harrison for supplying us with *Drosophila* of different strains and for examining some of our strains. Mr M. J. Healy has examined our data and helped us very materially by advice on the statistics used and in the expression of our results. We wish to express our sincere thanks to him. We are obliged to Mr A. J. Arnold for making the spraying apparatus used in the course of our work.

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THE EFFECT OF REPEATED SPRAYING OF INSECTS IN INCREASING THEIR RESISTANCE TO INSECTICIDES

II. THE EFFECT OF CARBON-DIOXIDE SENSITIVITY ON THE TOXICITY OF DDT WITHIN A STRAIN OF *DROSOPHILA MELANOGASTER*

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(With 4 Text-figures)

During the selection of a stock of *Drosophila melanogaster* for resistance to DDT, in which carbon dioxide was used for purposes of anaesthesia, a sensitivity to this gas developed. The phenomena closely paralleled those shown by the CO₂-sensitive ebony stock isolated by L'Héritier and his co-workers. An experimental analysis of its effect upon DDT sensitivity was made. It was found that a stock selected for CO₂ resistance gave the same probit regression line as the original stock. A CO₂-sensitive stock, whether anaesthetized with nitrogen or carbon dioxide, gave the same regression line at a temperature of 25° C. at which CO₂ sensitivity disappeared, or at 15° C. if adjustment to the proportion of deaths in the control was made. The effect of CO₂ was therefore to limit the population from which selection is made for DDT resistance, rather than to alter the distribution of DDT resistance within the stock.

INTRODUCTION

During the experiments described in Part 1 (Tattersfield, Kerridge & Taylor, 1953) it was observed that, after a period in which carbon dioxide had been used as an anaesthetic to produce quiescence during spraying, a number of insects were killed by the gas. This sensitivity was subject to considerable fluctuations, on many occasions few insects being affected, while on others there was a most pronounced mortality. The effect was not due to an impurity in the gas, and occurred whether it was prepared from marble and hydrochloric acid or derived from a cylinder, whether washed or unwashed, the only impurity being a small amount of air. It was early observed that the effect varied from day to day and with the time of day, a higher mortality occurring in the morning than in late afternoon; it was usually more marked on cool overcast days than on warm bright ones. The effect was not produced on a stock of insects known as the Rothamsted strain, nor with certain hybrids, kindly supplied by Mr Harrison of the John Innes Research Institution. It seems to have been associated uniquely with the stock of insects we were using, which had been captured in a waste box, probably containing rotten fruit discarded from a small orchard. It was a large colony and was cultured for a number of months before use in our first experiments in November 1949. Our aim had been

to use such a wild colony for our purposes as giving more scope for selection. It is referred to as our original strain and given the letter *O*, but it must be carefully distinguished from the Rothamsted stock derived from the John Innes Institution.

L'Héritier and his co-workers (L'Héritier & Teissier, 1937; L'Héritier, 1948, 1949*a*, *b*, 1951) discovered an ebony strain of *D. melanogaster* which showed CO₂ sensitivity to a marked degree, and its transmission, inheritance, selection and cure, have been very fully investigated. There are similarities between its inheritance and that of the cytoplasmic factors of the 'killer' phenomena in varieties of *Paramecia* but the parallelism is not exact, since 'no major influence of the genes on CO₂ sensitivity has ever been demonstrated'. L'Héritier summarizes the conclusions arrived at by himself and his co-workers in several valuable reviews (1948, 1949*a*, *b*, 1951); they should be seen in full for an appreciation of the significance of the phenomenon.

In discussing the fundamental aspects of this phenomenon, L'Héritier analyses in some detail the evidence in favour of the sensitivity to CO₂ being due to a virus disease or to cytoplasmic inheritance. He prefers to leave the issue open and to conclude that 'no advance will come from classifying the CO₂ genoid in any definite category, since its classification is doomed to remain so much a question of definition and personal feelings'. He suggests very cautiously that from a physiological point of view, the toxic effect of CO₂ may be localized in the thoracic nerve ganglion.

This important series of researches is of much interest to us, in view of the fact that, shortly following our initial selection sprayings with DDT using CO₂ for purposes of anaesthesia, our wild colony of *D. melanogaster*, found near the Rothamsted Laboratory in late 1948, showed a remarkable series of reactions. The first series of tests, made in November 1949 with the object of choosing appropriate concentrations of DDT to be used in selection, showed nothing abnormal in the behaviour of the flies. But some months later, particularly on cool days, application of CO₂ induced an abnormal death-rate. The CO₂ was prepared from pure marble and pure hydrochloric acid, and well washed in a solution of bicarbonate of soda. It was at first considered that probably hydrochloric acid gas was being carried over, but CO₂ from a cylinder, guaranteed to contain no impurity but a little air, had a similar effect. A low temperature favoured the effect and for some time this was regarded as the probable prime cause, and raising the temperature above 20° C. undoubtedly tended to eliminate the trouble. Nevertheless, considerable rises in the mortality rates led to a further investigation, and a sensitivity to CO₂ was apparent. Prof. L'Héritier kindly examined our insects and confirmed our results, and much of our later work has been concerned with the inter-relation of CO₂ sensitivity and the development of DDT resistance through selection spraying.

For some months the reason for the appearance of CO₂ sensitivity was quite obscure, but a certain reluctance to accept the view that a new stock of CO₂-sensitive *Drosophila* had arisen led to an investigation of the ultimate origin of the stock used in our experiments, and it was found that from 1942 to 1944 (when they were taken

away), stocks of L'Héritier & Teissier's ebony CO_2 -sensitive strain had been reared in Rothamsted, and the probability of insects escaping is by no means a remote one. If so, they must have crossed with one or more other strains and the 'ebony' character must have been lost, as no 'ebony' has been noted in our CO_2 -sensitive stock. They must have bred in the wild state for 4 years, and survived the severe winter of 1946-7, since they were collected as a large wild colony in 1948 (autumn). Further investigations may be necessary, but for the time being the above hypothesis for the appearance of CO_2 sensitivity in our stock seems the most likely but cannot be established beyond doubt.* Prof. L'Héritier has supplied us with his ebony stock, and its behaviour and reactions are being studied.

On the discovery, a decision had to be made whether further work on selection would be profitable. It was finally decided to continue as before with our DDT-selection spraying and its outcome, and to attempt to ascertain the effect if any, of CO_2 sensitivity on the selection for DDT resistance and vice versa, in the hope that it might shed some light on the inter-relations of biological and chemical control when applied successively or together, and their mutual bearing on the selection of resistant strains. It seemed not unlikely that the two effects might be correlated, particularly in view of the fact that the application of DDT to insects gave rise to a marked increase in the respiratory rate (Lord, 1949), and thus to the output of CO_2 .

Unfortunately we were unable to give close attention to the subject at the time of the highest degree of sensitivity to DDT in our cultures; nevertheless, the results obtained are of some interest, and it is noteworthy that, whereas in our original stock the sensitivity to DDT tended to decline towards the end of our experiments, the CO_2 sensitivity showed no such effects and was, if anything, greater in the later, than in the earlier experiments.

EXPERIMENTAL

A study was made of the sensitivity to CO_2 of the original wild stock used in our DDT selections, as contrasted with the strain known as 'Rothamsted' in the John Innes Research Institution collection. The insects were transferred to small tubes closed at both ends with nylon, and exposed at a temperature of 15°C . to a stream of CO_2 . The results are shown graphically in Fig. 1a: an exposure of 7.5 sec. accounted for a mortality in our sensitive stock of 55%, and 15 sec. for over 80%, whereas the John Innes 'Rothamsted' stock of *D. melanogaster* withstood with comparatively little loss an exposure of 240 sec.

Fig. 1b shows that, for both males and females, tests carried out in the morning gave a rather higher death-rate than those in the afternoon. Reference to Fig. 1d indicates that this effect was almost certainly associated with temperature, the temperature before treatment being apparently more important than the temperature subsequent to it. The implication is that the capacity for heat of the insect body

* Since this paper was sent in for publication Professor L'Héritier has (*in litt.*) informed us of the occurrence in France of wild stocks of *D. melanogaster* sensitive to CO_2 .

is such that it retains sufficient for some minutes to be able to resist the chilling effect of the stream of gas at a lower temperature (16–17° C.) and so overcome the negative temperature coefficient, shown in Fig. 2. Fig. 1c contrasts the effects of the two gases, CO₂ and N, the latter gas having little or no effect, a result which led to the adoption of N for anaesthesia in later experiments.

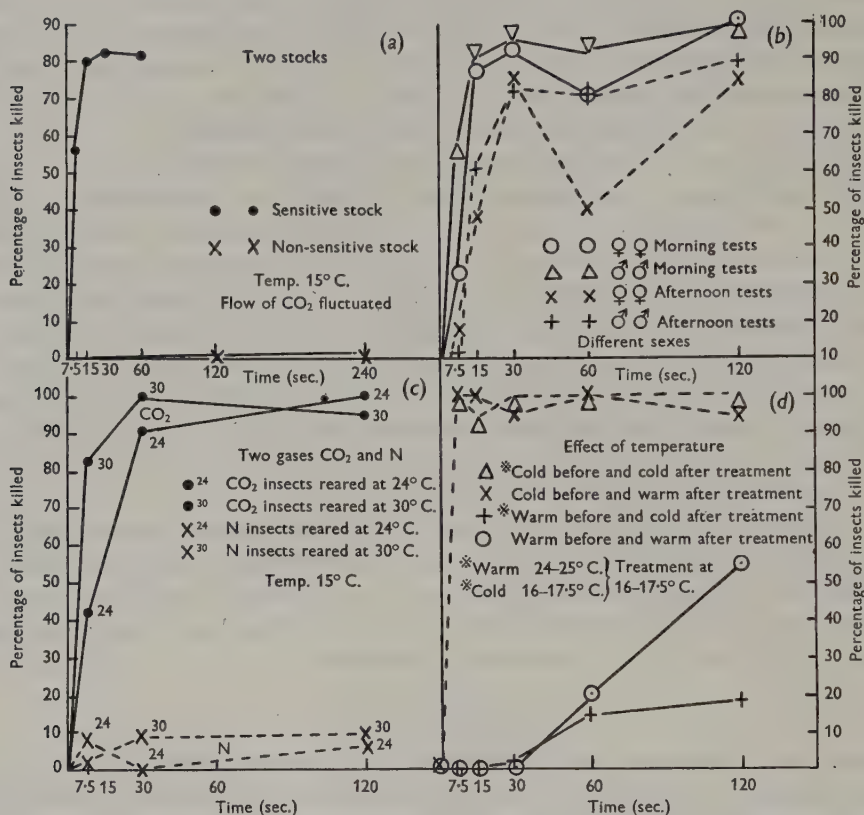


Fig. 1. The lethal effect of carbon dioxide on *D. melanogaster*. Approximate rate of flow 4.4 ml./sec. at NTP.

It is clear from these preliminary graphs that there is some fluctuation in sensitivity. A good deal of this is due to small variations in the temperature of the gas stream, and there is some indication that the temperature of rearing may influence mortality, but there is a strong likelihood that, within our strain, the number of sensitive individuals in cultures may vary with the culture. This is shown in Fig. 2c where in a test using insects not treated with DDT there is a mortality lower than that expected from the data presented in a and d of this figure, the differences in temperature of 2.5° C. being inadequate to account for the difference in mortality.

It has been known for some time that the effect depended on temperature, L'Héritier & Teissier (1937) showed that it was accentuated with decline of temperature but reached a minimum at 2.5° C. Above 23° C. the effect also disappeared with their strain. Our experience suggests that, although above 23° C.

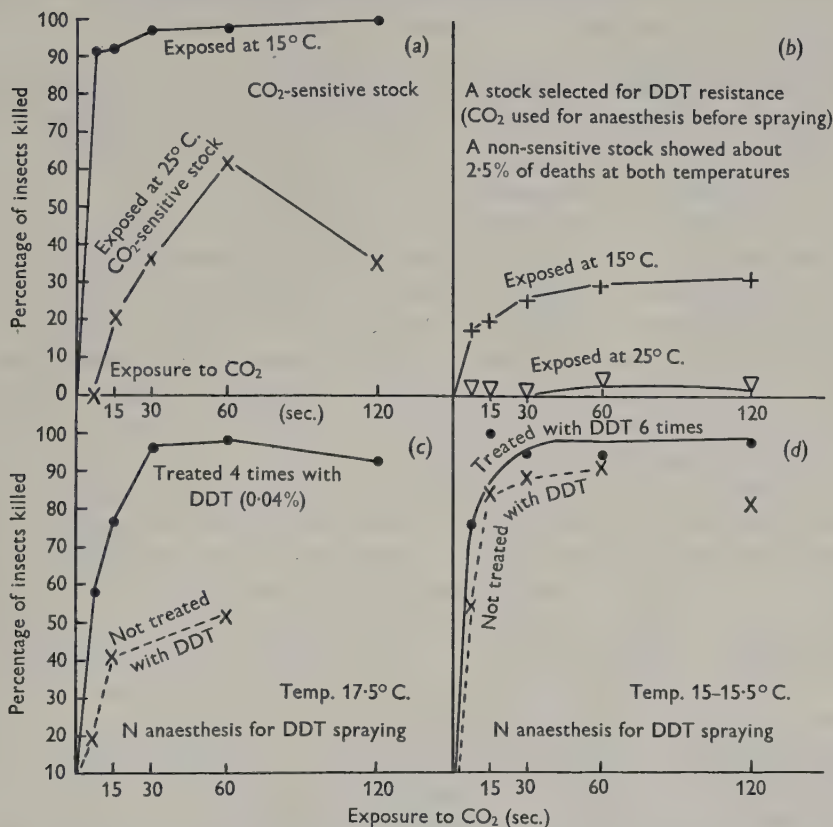


Fig. 2. Effect of temperature and of successive treatments with DDT on the CO₂ sensitivity of a CO₂-sensitive stock of *D. melanogaster*.

the effect may be small and in some cultures vanish altogether, nevertheless with our strain in a number of cultures the effect can be pronounced. This is illustrated in Fig. 2a. Although at 25° C. with this particular culture the effect is smaller than at 15° C., it is nevertheless produced. The effect may, however, be accentuated by the pressure of the flow of gas being slightly above the atmospheric. The discrepancy suggests that it may depend on the degree of infection with or even on the strain of the virus, the nature of the strain of *Drosophila*, or any combination of these. The first suggestion, at the moment, appears the more pertinent, but it may not account entirely for the fluctuations observed in the degree of sensitivity.

Fig. 2*b* illustrates the results obtained with the selected strain treated repeatedly with DDT (0.01%). The effect of both at 15 and 25° C. is markedly less than with the original strain, practically disappearing at the latter temperature. It must be borne in mind, however, that CO₂ had been used repeatedly for anaesthesia before spraying, and also that selection for CO₂ resistance is readily made. In view of this result, it became important to ascertain what the mutual effect of DDT and CO₂ selection might be.

A number of successive sprayings with 0.04% colloidal DDT were made, using N for anaesthesia, and after the fourth and sixth spraying the effect of CO₂ was ascertained. The results are shown in Fig. 2*c, d*. There was no marked degree of selection for DDT resistance noted after six sprayings.

The results plotted for the DDT-treated flies in Fig. 2*c* should be compared with those obtained in Fig. 2*d*, as it is clear that the CO₂ sensitivity of the control (unsprayed) stock was at the time of these tests apparently at a low level. It is clear that the treatment with DDT has little, if any, effect upon CO₂ susceptibility. In view of the slow process of selection for DDT resistance, the reverse process for selection for CO₂ resistance, much more readily achieved, was resorted to and the log concentration-regression line for DDT determined. The data are shown in Fig. 3. In these figures the number attached to the points or circles indicates the number of generations subjected to CO₂; ●° or O° implies that no previous treatment had been made, while ●² or O² would indicate that two treatments with CO₂ had been made before the test. Fig. 3*a* shows how rapidly the susceptibility to CO₂ can be eliminated, at least temporarily. After one treatment with a stream of CO₂ flowing at an approximate rate of 4.4 ml./sec. at N.T.P., a considerable amount of resistance has been built up, and after three or more previous treatments CO₂ sensitivity has practically disappeared, but whether a reversion would take place, and how rapidly, has not been ascertained by us.

In the tests summarized in Table 1 and Fig. 3, N was used for anaesthesia before spraying, and this procedure was adopted in all subsequent tests, except where comparisons were to be made between the effects produced by CO₂ and N in the mortality caused by spraying with DDT. The temperature was 19.5° C. There was some heterogeneity in the data for the *O* series treated with CO₂ and this had to be accounted for in the variances. It was considered advisable to use the factor determined for all the *O*'s in the analysis of χ^2 . A number of tests were made also with the *A* series treated with CO₂ three and four times, completely resistant to CO₂, but unfortunately a sufficient number of insects was not available for the *A* series untreated by CO₂. It is clear, however, that the *A* series untreated for several months was still significantly more resistant to the effect of DDT than the original stock. It does not seem at all probable that its susceptibility to DDT had been affected one way or another by selection for CO₂ resistance.

As far as the *O* series is concerned, the statistical analysis of the data given in Table 1 shows that there is some variation in the value of the log L.C. 50 (*m*)

and in the slopes of the individual regression lines, but this seems to be more or less at random, and the difference between the values for m runs in the order $O^5 > O^3 > O^0 > O^4$, the differences in no case being significant nor do the slopes

TABLE I. *The effect of selection for CO₂ resistance upon susceptibility to DDT. N anaesthesia*

Summary of results—deaths in the controls allowed for

Series and no. of CO ₂ treatments	log L.C. 50 mg./1000 c.c. DDT (m)	Slope (b)	χ^2 (3 D.F.)
A_3	2.404 ± 0.022	2.86 ± 0.30	4.02
A_4	2.426 ± 0.019	3.41 ± 0.30	7.46
O_0	2.246 ± 0.038	3.79 ± 0.71	6.71
O_3	2.283 ± 0.040	3.65 ± 0.75	18.67**
O_4	2.232 ± 0.047	3.27 ± 0.75	22.76**
O_5	2.321 ± 0.045	3.94 ± 0.92	16.23**
Combined A 's	2.416 ± 0.014	3.14 ± 0.21	15.92 (8 D.F.)
Combined O 's	2.273 ± 0.021	3.64 ± 0.38	75.09** (18 D.F.)

** $P < 0.01$.

Analyses of χ^2

A series	D.F.	χ^2	M.S.
Differences between slopes	1	1.67	—
Differences between m 's (common slope)	1	2.77	—
Residual	6	11.48	—
Total	8	15.92	—
O series			
Differences between slope	3	1.13	0.38
Differences between m 's (common slope)	3	9.59	3.20
Residual	12	64.37	5.36
Total	18	75.09	4.17

A combined heterogeneity factor of 5.36 is applied to all the variances in the O series. Omitting the lowest dose in each series and neglecting the deaths in the controls (which do not exceed 3.7%) reduce the heterogeneity factor to 3.56 without altering the general conclusions. Within each series neither the slopes nor the log L.C. 50's differ significantly. On combining the data for each series, the A series (selected for DDT resistance) is still significantly less susceptible to DDT than the O series. The difference between the log L.C. 50's is 0.143 ± 0.025 . The A series is 1.4 times as resistant as the O series.

differ significantly. The regression line for the combined data was therefore calculated and is plotted in Fig. 3*b* along with that for the A series. The two mean lines differ significantly in the value for m , the median lethal concentration for the A series selected for DDT resistance is still 1.4 times that of the O series, the original unselected series. The data indicate that selection for CO₂ resistance has little, if any, effect on the susceptibility of the adults of our strain of *D. melanogaster* to DDT.

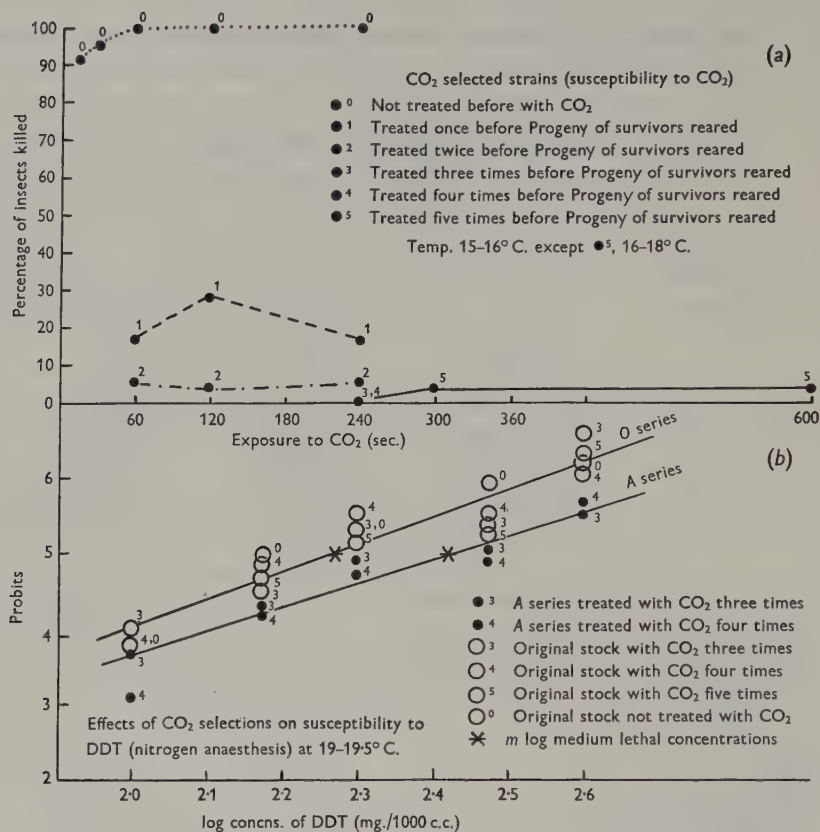


Fig. 3. Effect of successive breeding from survivors of *D. melanogaster* (CO₂-sensitive stock), after treatment with CO₂, upon susceptibility to CO₂ and DDT.

CO₂ and N compared, when used for anaesthesia, in their effect on susceptibility to DDT

For these experiments the original stock of *Drosophila* sensitive to CO₂ was used at two widely separated temperatures. One, 25° C., was chosen because the effect of CO₂ was almost nil at that temperature, the deaths in the controls sprayed with the carrier, being for CO₂ 2.0%, and for N 2.4%; the other 14.5–16.5° C. because the effect of CO₂ was large; in our experiments the deaths in the control spraying reached a level of 53% with CO₂, while in the nitrogen control the mortality of 1.82% was of the same order as that shown at 25° C.

The tests were done on the usual lines of alternate sprayings carried out as rapidly as possible, the insecticide being suspended in an acetone-sulphonated lorol medium containing throughout 2.5% acetone and 0.05% sulphonated lorol. The appropriate gas (CO₂ or N) was used for anaesthesia in sorting and counting

out and also just before spraying. Neither of the two gases was used again, etherization being employed for the examination 2 days following treatment.

The results are set out in Table 2 and Fig. 4.

TABLE 2. *Effect of CO₂ and N anaesthesia upon susceptibility to DDT of adult Drosophila melanogaster of a CO₂-sensitive strain, at two temperatures*

Date and temperature	Series	log L.C. 50 (<i>m</i>)	Slope (<i>b</i>)	% control mortality	χ^2
6. xii. 51, 25.5–26° C.	CO ₂	2.433 ± 0.017	3.32 ± 0.35	2.0	3.00 (2 D.F.)
	N	2.430 ± 0.028 ± 0.016	3.54 ± 0.59 ± 0.34	2.4	6.15*† (2 D.F.)
	Combined	2.431 ± 0.014	3.43 ± 0.08	—	9.35 (6 D.F.)
8. iv. 52, 14.5–16.5° C.	CO ₂	2.337 ± 0.022	4.93 ± 0.62	53.2	0.26 (3 D.F.)
	N	2.367 ± 0.011	4.77 ± 0.30	1.8	7.50 (3 D.F.)
	Combined	2.359 ± 0.010	4.87 ± 0.27	—	9.55 (8 D.F.)
	Combined with extra points†	2.359 ± 0.009	4.92 ± 0.26	—	5.35 (12 D.F.)

* $P < 0.05$.

† χ^2 for this series is just significant at the 5% level. The upper standard errors are derived with a heterogeneity factor of 3.07, the lower without allowance for heterogeneity.

‡ The extra points were the results of doses of 300 and 200 mg./1000 c.c. DDT applied to insects of the same stock selected seven times for CO₂ resistance.

The probit log concentration regression lines given in Fig. 4*b, c* illustrate the importance, in experiments of this kind, of allowing for the deaths taking place in the controls. When the appropriate allowance for these control mortalities is made, the data for both gases fall on one line without heterogeneity (Fig. 4*a*). In addition, four points marked Δ and O in which flies had been selected for CO₂ resistance fall on the same line irrespective of CO₂ or N being employed. Where no allowance is made for the controls, as shown in Fig. 4*b*, the regression line of probits upon the log concentration of DDT, in the case of CO₂ anaesthesia of the original stock, is curved and stands well to the left of the straight line representing the regression line for nitrogen anaesthesia and the four points of the CO₂-resistant strain. Fig. 4*c* represents the data accumulated for the sprayings carried out at 25.5–26.5° C. The small numbers of deaths in the control sprayings with the medium (2.0% for CO₂, and 2.4% for N) do not affect the conclusions and all the data for the regression of probits of percentage kill upon the log of the concentration of DDT can be represented without heterogeneity by one line, whether CO₂ or N is used for anaesthesia during spraying.

Above 25° C. the CO₂ effect largely disappears and it is clear it has no effect upon the toxicity of DDT and the resistance to it of the insect. However, the values obtained at approximately 15° C. when the CO₂ effect alone is sufficient to account for over 50% of deaths, show that the residue left after such treatment

gives a regression identical with the whole unselected population containing both CO_2 -susceptible and non-susceptible insects. Moreover, the values for insects selected by seven successive treatments with CO_2 , whether anaesthetized by CO_2 or N, fall on the same line. The tentative conclusion that CO_2 has no direct effect

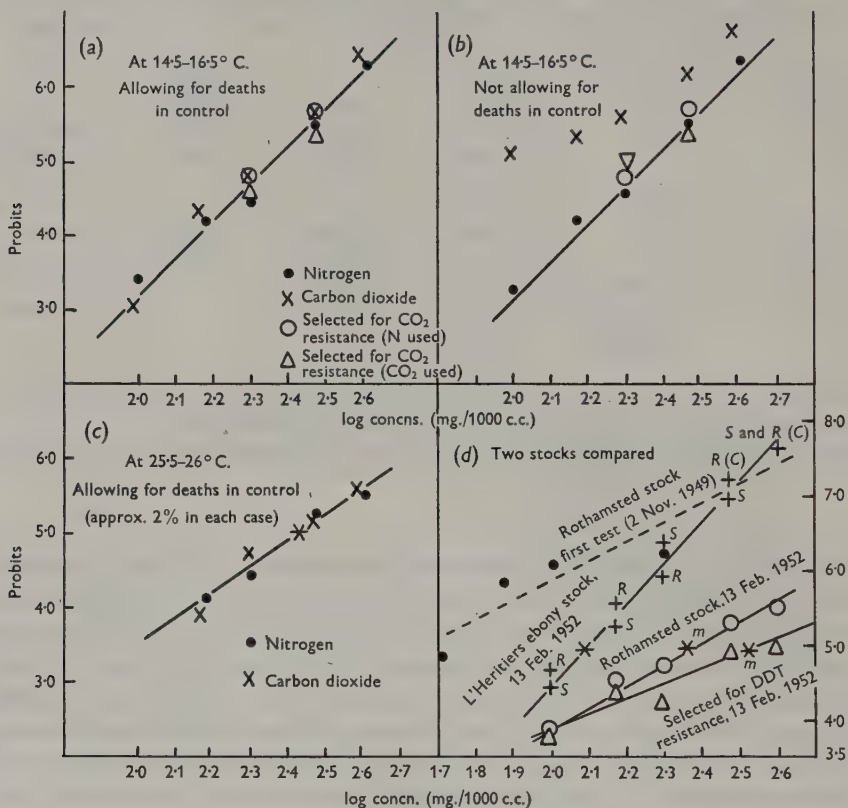


Fig. 4. Comparison of CO_2 and N as anaesthetics in their effect on toxicity of DDT to *D. melanogaster* (sensitive to CO_2). d: ---, approximate position of regression line for the first series of tests with DDT; ○—○, Rothamsted original stock after 2 years; △—△, a stock selected for DDT resistance but not sprayed for 8 months; +—+, L'Héritier's ebony stock—S=sensitive to CO_2 , R=not sensitive; + + (c), computed points; * *, median lethal concentration (log mg./1000 c.c.).

upon the susceptibility of these insects to DDT appears a safe one to draw, and that the distribution of DDT resistance in the CO_2 -sensitive portion of the population is the same as in the CO_2 -resistant portion. The role of CO_2 in these experiments appears to have been one of limiting the population upon which selection for resistance to DDT could act.

TABLE 3. *DDT resistance in different stocks of Drosophila melanogaster. N anaesthesia*

Series and CO ₂ sensitivity	log L.C. 50 (<i>m</i>)	Slope (<i>b</i>)	χ^2
13 February 1952			
<i>O</i> (susceptibility)	2.394 ± 0.022	2.81 ± 0.29	1.53 (3 D.F.)
<i>A</i>	2.540 ± 0.045	1.91 ± 0.30	1.91 (3 D.F.)
L'Héritier's ebony			
Susceptible	2.094 ± 0.014	6.14 ± 0.49	6.14 (3 D.F.)
Resistant	2.077 ± 0.019	4.80 ± 0.50	4.80 (3 D.F.)
Combined	2.087 ± 0.012	5.49 ± 0.35	15.13 (8 D.F.)
7 July 1952			
<i>O</i> (susceptible)	2.224 ± 0.012	4.55 ± 0.30	3.77 (4 D.F.)
L'Héritier's ebony			
Susceptible	2.172 ± 0.011	5.95 ± 0.39	8.19 (4 D.F.)

In the first test, *A* is significantly more resistant than *O*, and *O* is more resistant than ebony, comparing the strains at the 50% level of kill. At this level the lethal doses are in the ratio 2.8:2.0:1.

In the second test, *O* is significantly more resistant than ebony at the 50% level of kill, the lethal doses being in the ratio. 1.1:1.

The susceptibility of L'Héritier's ebony strain to DDT

The data in Table 3 and the regression lines in Fig. 4*d* show that the *A* stock, despite no further selection having been made, has maintained its greater resistance to DDT over the original stock. The slopes of the two lines are just significantly different and thus the difference in resistance to DDT becomes somewhat greater at higher mortalities. The differences between the values for *m* and in the 'slope' for these two stocks and for the ebony series are noteworthy. The data for the two ebony strains can be represented by one line without heterogeneity, since neither the values for *m* nor for parallelism show significance. At the median lethal concentration the *O* series is about twice, and the *A* stock 2.8 to 2.9 times as resistant to DDT as the ebony stock, whether sensitive to CO₂ or not. The difference in the slopes of the two ebony stocks is not significant, but it approaches significance, a result which may well be due to chance. Fluctuations in the values of *m* and of 'slope' of a high order may well be characteristic of unselected stocks, as is illustrated by a comparison made at a still later date (7 July 1952) of the sensitivity to DDT of the two CO₂-sensitive stocks—our original and L'Héritier's 'Ebony'. The data, together with those shown in Fig. 4*d*, are also given in Table 3. The slope for the ebony stock is of the same order some 5 months later, but the value for *m* has increased slightly; the slope for the *O* stock, however, is much greater and commensurate with it there has been a distinct fall in the log L.C. 50 (*m*). The ebony strain, although it has a steeper slope, approximates in DDT susceptibility to the original 'Rothamsted' stock in the early stages of our experiments on its resistance to DDT, but this, apparently, is not associated with CO₂ susceptibility.

DISCUSSION AND CONCLUSIONS

In the series of experiments undertaken to ascertain whether successive spraying with suspensions of DDT of the adults of a wild colony of *Drosophila melanogaster* would lead to the development of resistance to the insecticide, CO₂ was used for anaesthesia necessary for counting the insects, and to secure quiescence during the actual spraying. After a period, sensitivity to this gas developed and the insects began to display many of the properties shown by L'Héritier's ebony CO₂-sensitive strain of *Drosophila*.

This ebony strain had been cultured at Rothamsted some years before the capture of the wild colony used in these experiments and, although conditions had not been very favourable to its survival, it is possible that escapes and crossing with other strains had ensued giving offspring retaining the potential sensitivity. This character, however, would have had to endure for a period of years (about four), and alternative hypotheses for the appearance of CO₂ sensitivity are not ruled out. L'Héritier ascribes the CO₂ sensitivity of his ebony strain to a virus, or to a plasma-gene or 'genoid', showing characteristic hereditary features which he and his co-workers have worked out in detail.

In our observations the sensitivity increased with time and showed a characteristic negative coefficient of intensity with temperature, but so far we have not been able to correlate the effect with any physiological characteristic associated with the insecticidal potency of DDT. It was, however, of some interest to ascertain the mutual influence of the two kinds of sensitivity and the effect of selection for DDT and CO₂ resistance. The problems can be put under the following heads: (1) does the selection for DDT resistance have any effect on CO₂ sensitivity? (2) does the selection for CO₂ resistance have any effect on DDT sensitivity? (3) what is the effect of a sensitivity to CO₂ of the type shown by this stock of insects, upon insect control by insecticides and its measurement?

(1) During the course of the experiments upon the selection by successive sprayings of DDT-resistant strains, a stock (*A*) of *D. melanogaster* had been built up with a higher resistance to DDT than that of the original; its sensitivity to CO₂, although distinctly noticeable, was also less than that of the original stock, but CO₂ had been repeatedly used during the course of these experiments, and the probability that selection for CO₂ resistance by its use had taken place is considerable. In addition, towards the latter part of our experiments the non-selected original stock itself showed signs of increased resistance to DDT; nevertheless, the stock was more sensitive to CO₂ than in the early stages of our work. Experiments in a fresh selection for DDT resistance by repeated sprayings, in which N was used for anaesthesia were continued for six generations of the original stock; the amount of selection for DDT resistance was not very marked in that time, but, if anything, the CO₂ sensitivity was slightly accentuated. Our experiments do show that DDT resistance is not necessarily correlated with resistance to CO₂.

(2) As selection for CO_2 resistance is speedy when contrasted with that for DDT, a series of selections on our original stock were made, varying from 'no treatment' up to five treatments with CO_2 . Three treatments practically eliminated any sensitivity to CO_2 . These cultures were examined for DDT sensitivity along with the *A* stock (DDT-resistant), also treated three and four times with CO_2 , by a determination of the probit-log concentration regression line. The *A* stock was still more resistant than the original stock to DDT. It was found that one regression line would represent the data for the original stock unsprayed with DDT, whether it had been subjected to selection for CO_2 resistance or not. The values for m (log L.C. 50), for the whole set were distributed at random round the mean and did not differ significantly; neither did the slopes of the lines. The conclusion to be drawn was that selection for CO_2 resistance had had no significant effect upon DDT resistance.

Although no test was possible on the *A* stock untreated by CO_2 , the *A* and *O* stocks subjected to CO_2 treatment three and four times were still significantly different from each other in resistance to DDT, and it seems highly improbable in their case that selection for CO_2 resistance has affected the DDT resistance of the *A* stock.

Two critical experiments at the two temperatures of 15 and 25° C. were undertaken. At the lower temperature the CO_2 effect is pronounced, but it tends to disappear at 25° C. The original stock (CO_2 sensitive) was used in determining a log concentration regression line in which CO_2 and N were respectively used for anaesthesia. At the higher temperature only 2% of insects were killed by each gas, but at 15° C. CO_2 accounted for over 50% kill in the control sprayings with the carrier, compared with 1.8% with N. At the higher temperature the values for the log median lethal concentrations were identical, and the slopes of the lines did not differ significantly; the data for both gases could without heterogeneity be represented by one line. The data for the two gases at the lower temperature were markedly different, when taken *without* any correction for the kills in the control sprayings. The application of the usual correction for control kills, however, made both lines practically identical, and the data for both could, without heterogeneity, be represented by one line, and in addition this line fitted four points representing the results for the same stock but selected for CO_2 resistance, irrespective of CO_2 and N being used for anaesthesia.

The effect therefore of the administration of CO_2 in a CO_2 -sensitive stock with subsequent selection for DDT resistance appears to be one of limitation of the population from which the selection is made, since the population of insects of which 50% have been eliminated by CO_2 administration have the same regression upon resistance to DDT as the unselected population. In so far as this would reduce the scope of selection the incidence of a virus might slow down the appearance of highly resistant stocks, but it would not inhibit their appearance altogether.

(3) It would appear, therefore, as if disease of an insect test subject might pro-

foundly affect its control, particularly under conditions of maximum sensitivity to it, but the assessment of the toxicity of an insecticide, if superimposed upon the effects of disease, would have to be made independently, and after due allowance had been made for the mortality in the controls due to the infection, unless it could be demonstrated that the incidence of the disease affected and accentuated the physiological action of the insecticide.

We wish to express our thanks to Dr C. Potter for constructive criticism and advice, and to recognize our great indebtedness to Mr M. J. Healy for much valuable advice upon the statistical side of our work, and for checking and rearranging our tables of data. To Prof. Ph. L'Héritier we are indebted for examining our CO₂ sensitive strain of *Drosophila* and for a gift of his ebony strain.

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STUDIES ON THE FEEDING OF *MYZUS PERSICAE* (SULZ.) ON RADIOACTIVE PLANTS

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(With 2 Text-figures)

Adult apterae of *Myzus persicae* (Sulz.), were fed, after a period of fasting, on leaves containing radioactive phosphorus. The weight of sap imbibed by the aphids after varying feeding times was estimated by relating their radioactivity, at the end of each feeding period, to the activity per unit fresh weight of the leaf lamina on which they fed. The calculations were made on the assumption that ^{32}P is uniformly distributed in the leaf tissues.

The mean rates of uptake so estimated were about 10 $\mu\text{g.}$ of sap for the first hour of feeding; 40 $\mu\text{g./hr.}$ between 1 and 4 hr. feeding, and 17 $\mu\text{g./hr.}$ between 6 and 24 hr. feeding. The decrease in apparent rate of uptake with the longer feeding times is attributed to loss of ^{32}P in nymphs born during the feeding period.

When aphids were fed on seedlings raised in water culture solution containing ^{32}P no activity was detected after 5 min. feeding and an insignificant fraction after 15 min., but when the isotope was introduced by immersing the leaves for several days in the culture solution, aphids fed for 5 and 15 min. became appreciably active.

The increase in rate of uptake after 1 hr. of feeding indicates that aphids do not start to feed normally until they reach the phloem, but the activity after short feeding times suggests that previously starved aphids feed to some extent on other tissues, possibly only on the epidermis.

INTRODUCTION

The study of aphid feeding offers an approach to many unsolved problems of virus transmission. One of us (Hamilton, 1935), attempted to measure the feeding rates of *Myzus persicae* (Sulz.), using polonium as a radioactive indicator, but the use of artificial feeding methods, necessitated by failure to introduce polonium into living leaves, meant that the results were subject to large errors, and probably bore little quantitative relation to natural conditions. Hamilton estimated the quantity of liquid taken up by aphids feeding normally on living leaves by weighing a known number of aphids, starving them for a time and then measuring the gain in weight during subsequent feeding. The average weight of an individual *M. persicae* was about 500 $\mu\text{g.}$, falling to 400 $\mu\text{g.}$ on starving in humid conditions for about 14 hr.; the gain in weight after feeding for 6 hr. was about 80 $\mu\text{g.}$ The method suffers from the obvious disadvantage that it takes no account of loss by evaporation and excretion, and no correction was applied for young born during the test feeding. The wide range of radioactive isotopes now available, together with more refined techniques for measurement of radioactivity, seems to offer an opportunity for

better work of this kind; it would be particularly valuable if the sensitivity were adequate to determine quantities imbibed by aphids in feeding times less than 10 min., because these times are of great importance in the transmission of non-persistent viruses. As with other insects (Flemion, Weed & Millar, 1951; Day & McKinnon, 1951), aphids fed on leaves of plants which have received part of their phosphorus as the radioactive isotope, ^{32}P , become radioactive, and their activity increases with increasing feeding time. Relating the activity of the aphid after feeding to the activity per unit fresh weight of the leaves upon which they fed, gives an estimate of the rate of uptake of sap. However, the interpretation of the results will depend upon whether availability of ^{32}P to the aphids varies in different conditions of the growth of the plants or in different tissues of the leaves. For most of this paper, the 'uptake' referred to is really apparent uptake based on the supposition that the ^{32}P is equally available to the aphids at all times. The results themselves indicate that this is not true, but they agree sufficiently with estimates made by other methods, and are sufficiently consistent between different experiments to provide a reasonable working hypothesis about the quantities of plant material imbibed by aphids and the changes in rate of uptake which take place with time.

METHODS

Two methods of introducing ^{32}P into the food plants were used. For Exps. I, II and III, turnip and sugar-beet seedlings were raised in sand culture and transferred in the cotyledon stage to water culture in 120 ml. bottles. When the first leaf was fully developed ^{32}P as H_3PO_4 was added to the culture solution in known amounts. The activity of the leaves was roughly determined by daily readings with an end-window counter attached to a rate-of-count meter. According to these readings the turnip leaves reached their optimal activity after about 4 days, and sugar beet after about 7 days, but increase in leaf area probably affects counter geometry and such counts are subject to large errors. With both plants apparent activity remained near the initial high level for several days, during which time the feeding experiments were made. The seedlings were enclosed in individual transparent cages during treatment.

In Exp. I, made on turnip seedlings, ^{32}P was given on 25 March at 100 and $400\mu\text{C./l.}$ of culture solution; giving approximate atomic ratios $^{32}\text{P}/^{31}\text{P}$ of 1.5 and 6.0×10^{-9} respectively at the time of application. Plants receiving the higher level showed damage, about 3 weeks after application, comparable to that described by Russell, Adams & Martin (1949), but at the time of the experiments they appeared normal. Aphids were fed on two occasions, 28–29 March and 3–4 April on the same eight plants. One and 24 hr. feedings were made on the first occasion; 1, 4 and 24 hr. feedings on the second.

Exp. II was made with a new set of turnip seedlings which received ^{32}P at the rate of $300\mu\text{C./l.}$ on 25 April. The feeding treatments, replicated on six plants, were made on 1–2 May.

Exp. III was made on 4-5 May on sugar beet sown and treated at the same time as the turnips of Exp. I, but which had been given a second application of $150\mu\text{C./l. }^{32}\text{P}$ on 25 April.

The second method of applying ^{32}P to the food plants was used for Exps. IV and V. Turnip and tobacco plants were grown in potting compost until their leaves were 5-10 cm. long. Then leaves were detached and immersed for 3 days in culture solution with $300\mu\text{C./l. }^{32}\text{P}$, contained in shallow dishes covered with sheets of glass. The leaves were then removed, thoroughly washed in running water and dried with absorbent tissue. They were placed in moist chambers consisting of glass tumblers inverted over damp sand for aphid feeding on 13-14 May. Each feeding treatment was repeated on eight leaves.

The culture solution used throughout was 'solution X' of Franco & Loomis (1947). This has a low phosphorus content and was chosen to ensure that a high proportion of the radioactive phosphorus would rapidly reach the leaves.

All experiments were made with mature apterae, previously cultured on turnip plants and handled by the methods described for virus transmission. Groups of eight to ten aphids were used for each treatment, because individual aphids do not acquire sufficient activity in short feeding times for convenient counting, and also because preliminary tests showed that activity varies widely between individuals even when they have been fed for the same time on the same plants. Day & McKinnon (1951) found similar differences between uptakes by individual *Orosius argentatus* when fed on radioactive sources.

To provide an estimate of error each series of feeding treatments was repeated on six or eight seedlings or detached leaves. All aphids were starved overnight in cool moist conditions before feeding, and those given feeding times of from 5 to 15 min. were watched until they started to penetrate the leaves before being timed. After feeding the aphids were removed from the leaves, killed by exposure to chloroform vapour, and each group arranged on a sample tray under a collodion film a few microns in thickness. The aphids were then dried at 100°C. before the activity was measured with an end-window tube attached to a scaling unit. No correction was applied for radiation absorbed in the dried aphids or in the collodion film.

At the end of an experiment the leaves were weighed, dried at 100°C. and weighed again, and then brought into solution by boiling with sulphuric acid. After suitable dilution the activity of the solutions was determined in a jacketed counter tube. The relation between counts recorded from this tube and those from the end window tube used for the aphid assays was determined experimentally.

RESULTS

Table 1 summarizes the results of five experiments. For Exp. I each figure is the mean of four feedings made on two occasions with an interval of 6 days, and on plants supplied with two rates of ^{32}P . Two results in the 1 hr. feedings were rejected because they were anomalously high. Most $\frac{3}{4}$ and 1 hr. feedings showed apparent

uptakes normally distributed around $10 \mu\text{g.}$, but on three occasions (one in Exp. V), one figure was of the order of $100 \mu\text{g.}$ As this quantity is well outside the expected value and the distribution was not continuous, these results have been excluded as being possibly attributable to contamination, or to the inclusion by accident of an aphid which had fed for a much longer period. Means and errors for the complete data are given at the foot of Table 1.

TABLE 1. *Apparent weights of plant material, $\mu\text{g.}$ per aphid, ingested by Myzus persicae*

(1) Aphids fed on seedlings raised in culture solution containing ^{32}P

Test plant	Counts per min. per $\mu\text{g.}$ fresh wt. of lamina	Times of feeding on active leaves							
		<div> <div>1 hr.</div> <div>4 hr.</div> <div>24 hr.</div> </div>							
Turnip I	0.267	Occasion 1	13.9*		—		442		
		Occasion 2	9.6		131		474		
	0.845	Occasion 1	8.8		—		438		
		Occasion 2	7.4*		139		493		
		Mean	10.0 \pm 0.89		134 \pm 23.0		462 \pm 36.7		
		Times of feeding on active leaves							
Turnip II	4.577	15 min.	30 min.	1 hr.	2½ hr.	4 hr.	6 hr.	15 hr.	20 hr.
		0.3	4.4	—	77	—	151	320	365
		S.E. \pm 0.14	\pm 1.08		\pm 6.84		\pm 12.7	\pm 29.9	\pm 52.1
Sugar beet III	0.599	S.E.	—	—	10.2	—	20.8	—	55
					\pm 2.17		\pm 2.70		\pm 5.6

(2) Aphids fed on leaves previously immersed in ^{32}P solution

Times of feeding on active leaves									
Test plant		S.E.	5 min.	15 min.	45 min.	2 hr.	2½ hr.	15 hr.	24 hr.
Turnip IV	0.719	S.E.	2.8	3.6	9.1	38	—	613	—
			\pm 0.78	\pm 0.95	\pm 1.56	\pm 11.0		\pm 186	\pm 111
Tobacco V	0.498	S.E.	2.4	2.0	6.0†	—	62	—	621
			\pm 0.69	\pm 0.75	\pm 0.85		\pm 7.1		\pm 11.4

Occasion 1. Aphids fed on 28–29 April.

Occasion 2. Aphids fed on 3–4 May.

* One count rejected—if these were included the mean would be 20.7 ± 7.97 .

† One count rejected—if this were included the mean would be 17.6 ± 11.45 .

The activity of the aphids relative to that of the plants was independent of the level of activity of the plants, and of the time interval between occasions. The relationship therefore appeared to be reasonably constant in varying conditions.

In Exp. II the activity of the leaves was higher than in Exp. I, but the results give comparable estimates of uptake. When the two experiments are combined and the results plotted, they fall reasonably close to a smooth curve (Fig. 1). The mean rate of uptake, estimated by fitting a linear regression of ^{32}P content on time to the

combined results, was $19.8 \pm 1.13 \mu\text{g./hr.}$ However, inspection of the data suggested that the relation between uptake and time is not strictly linear but follows an S-shaped curve, the rate of uptake being initially low, increasing in an intermediate period and subsequently decreasing again. This can be shown by fitting separate regression lines for successive time intervals, giving regression coefficients for:

$$0-1 \text{ hr. feeding} = 10.50 \pm 1.25 \mu\text{g./hr.}$$

$$1-4 \text{ hr. feeding} = 41.62 \pm 5.34 \mu\text{g./hr.}$$

$$6-24 \text{ hr. feeding} = 17.01 \pm 2.91 \mu\text{g./hr.}$$

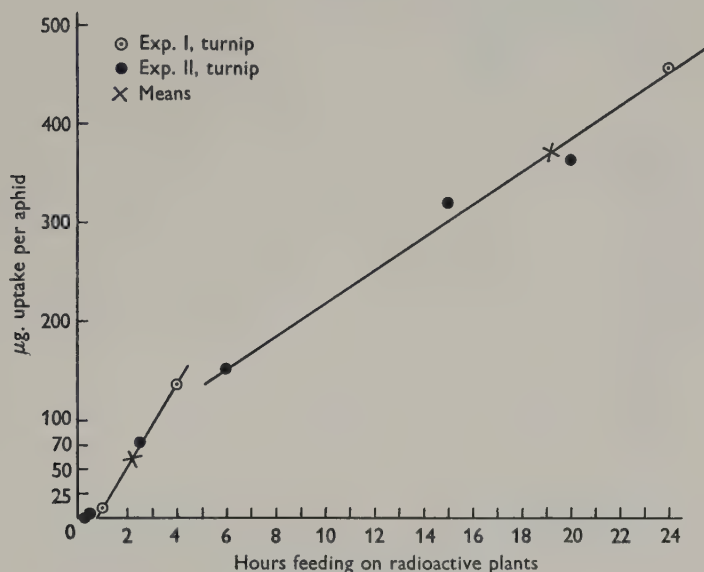


Fig. 1. Rate of uptake by aphids feeding on radioactive turnip, for times between 15 min. and 24 hr. Regression coefficients: 1-4 hr., $41.62 \pm 5.34 \mu\text{g./hr.}$; 6-24 hr., $17.01 \pm 2.91 \mu\text{g./hr.}$

In Exp. III the aphids were fed on detached leaves from sugar-beet plants which had been raised in the same way as the turnips in Exps. I and II, but were much older, as the aphids were fed 9 days after the second application of ^{32}P to the culture solution. The activity of the leaves was high, but the mean apparent uptake was small compared with the other experiments, and less than would be expected from other evidence. Possibly the radiation caused some damage which made the leaves unpalatable, or the ^{32}P may have become incorporated in solid plant tissue and was thus less available to the aphids.

In all these experiments and in a number of short feeding tests not recorded in Table 1, we failed to detect appreciable activity for feeding times of less than 30 min. There were two possible interpretations of this result. Either the aphids did not imbibe plant sap during the first 15-30 min. of feeding, or the sap which

they imbibed was not radioactive. Radioautographs of whole leaves and of microtome sections showed accumulation of radioactivity in the neighbourhood of the veins, but it was uncertain whether this concerned the whole vascular tissue or merely indicated the presence of the isotope in the xylem, passing from the culture solution into the leaf. In the sections, removal of the isotope from superficial tissues during fixation may also have contributed to the effect. However, there was a possibility that the phloem also contained a higher concentration than the rest of

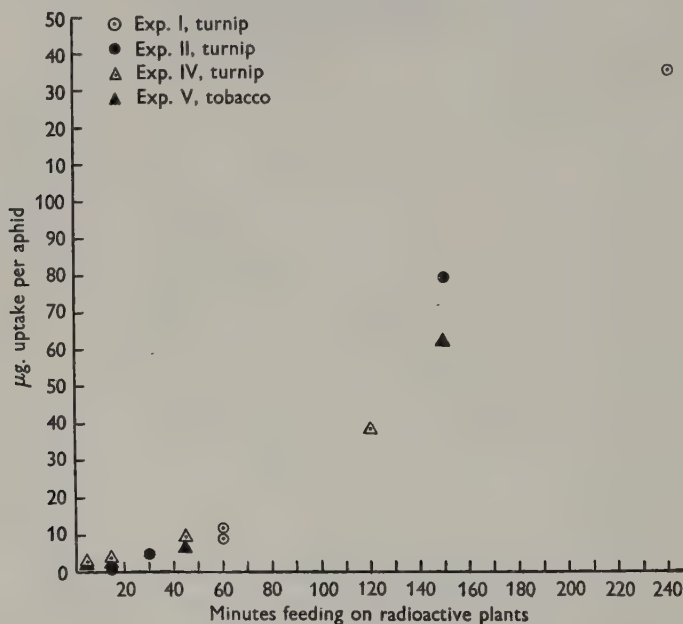


Fig. 2. Rate of uptake by aphids feeding on radioactive turnip and tobacco plants, for times between 0 and 4 hr.

the leaf. If this is so, the activity of the aphids after short feedings would be low, providing no information about the actual amount of feeding in superficial tissues. Exps. IV and V were therefore made with leaves which had been completely immersed in the active solution, and in which the presence of some ^{32}P in the superficial tissues could reasonably be assumed.

Aphids fed for 5 and 15 min. on both turnip and tobacco leaves exhibited measurable activity, though there was no difference between the two times. With this method there was danger of radioactive contamination of the aphids from the moist surface of the leaves. As a measure of this all aphids were confined on damp filter-paper for several minutes after feeding and before preparation for counting. The filter-papers were extracted with acid and the activity of the extract determined. Averaging over all the 722 aphids used in the experiments the count corresponded to 2.9 $\mu\text{g.}$ of sap per aphid. Probably aphids which feed for long periods become

more contaminated than those given very short times, and probably also, the filter-papers became contaminated by the brushes used to transfer the aphids. Aphids which were made to walk about on active leaves without being allowed to feed apparently became contaminated to the extent of about $1\text{ }\mu\text{g./aphid}$. If this represents the true amount of contamination during short feeding there is some suggestion that a very small amount of sap is imbibed between 0 and 15 min. feeding. There is little to indicate whether this is picked up immediately after penetration, or whether there is a very small but constant uptake during the first $\frac{1}{2}$ hr. of feeding.

The results from Exps. IV and V agree well with those for Exps. I and II, and again, especially for the short- and medium-length feeding times, the points fall reasonably closely to a smooth curve (Fig. 2), when the four experiments are combined.

In Exp. IV the uptakes for the longer feedings are very inaccurately determined. Taken at their face value both experiments show a higher average rate than previously. There is the same kind of reduction in the rate of uptake with longer feeding times as in the previous experiment.

This reduction in rate of increase of activity with longer feedings can be accounted for by the production of nymphs. Young born of parents which have fed on radioactive leaves are highly active, and their activity increases with increasing time spent by the parents on the radioactive source. After long periods some give counts which represent a high proportion of the activity of the parent. This is readily understandable from radioautographs made from serial sections of aphids fed for 24 hr. or more on radioactive leaves, for there is dense blackening in the developing embryos, showing that much of the phosphorus taken up by the parents goes to the germ tissue. In Exps. IV and V the average number of young produced during 15–24 hr. feedings was 1.3 per adult. Young born during feeding times between 1 and 6 hr. are inactive or only slightly active, so the rates of uptake indicated by increasing radioactivity of the adults during this period, i.e. 30–40 $\mu\text{g/adult/hr.}$, probably more nearly represent the normal constant rate of uptake than those for any other period of feeding on radioactive plants.

Tests were made during the earlier experiments to find whether ^{32}P were excreted by the aphids while feeding; these were mostly negative or showed only trace activity. Day & McKinnon (1951) show that, in some conditions, leaf hoppers may excrete considerable amounts of ^{32}P , and this was also observed by Flemion *et al.* (1951). However, in some of Day & McKinnon's experiments the isotope was not excreted so the evidence appears to be conflicting.

Possibly excretion of phosphorus depends partly on the amount and nature of the phosphorus compounds taken up by the insects, and is reduced in low phosphorus conditions such as those in which Exps. I and II were made.

If there is excretion of ^{32}P by aphids, and particularly if this varies with time, and with the conditions of feeding, the estimates of quantity of sap imbibed will be affected. However, errors are almost certainly introduced by the assumption that

the concentration of ^{32}P in the imbibed sap is the same as that in the rest of the leaf lamina. It seems unprofitable to attempt to correct the estimate of uptake for loss by excretion which is probably subject, independently, to the same kind of errors.

DISCUSSION

Assuming that ^{32}P is available to the aphids in epidermis and mesophyll, our data suggest that most aphids do not begin to feed normally until some time after penetration of the leaf surface. Roberts (1940) showed that when *M. persicae* feed on young turnip and sugar-beet leaves the stylets of about 10% reach the phloem in 15 min. and about 50% in 1 hr. It is reasonable to suppose that the increased rate of uptake after 1 hr. feeding shown in the present results, corresponds with the time at which the aphids reach the phloem, and that the S-shaped curve is an expression of the fact that different aphids reach it at different times.

The flattened region at the beginning of the curve suggests that either aphids feed at a very low rate in both mesophyll and epidermis, or else a few μg . of sap are quickly imbibed from the epidermis, but there is no further feeding until the phloem is reached. The experiments were made with starved aphids; unstarved aphids might behave differently with respect to this immediate uptake of sap.

The increased rate of feeding after the phloem is reached agrees with the rate of increase of infectivity of aphids transmitting persistent viruses such as beet yellows and potato leaf-roll viruses, but the rate of feeding seems to bear no relation to the transmission of non-persistent viruses. These are optimally transmitted by starved aphids after only about 2 min. feeding on infected plants. Roberts (1940) showed that it takes an aphid about 5 min. to penetrate through the epidermis. Therefore the non-persistent viruses are optimally transmitted from epidermal cells at a time when uptake appears to be small.

The hypothesis put forward by Sukhov (1944) and Bradley (1952), that the viruses are filtered out from the fluid imbibed by the aphids by the gelatinous sheath which is formed round the stylets might account for the loss of infectivity of aphids transmitting non-persistent viruses, but cannot explain the difference between persistent and non-persistent viruses. From what is known of their properties there seems no reason why one kind should be filtered out and the other allowed to pass through.

The manner in which non-persistent viruses are carried by the aphids is still uncertain. Bradley (1952) expresses the opinion that they are taken into the mouthparts, held there when feeding stops, and re-injected directly into the next cell penetrated.

If this is true, the exclusion of the virus by the stylet sheath could not account for the results unless the aphids feed on the mesophyll, because the virus would presumably remain in the mouthparts as long as the aphids do not feed. The loss of infectivity would then be slower than observed. On the other hand, if the aphids do not feed on the mesophyll, and if unstarved aphids do not feed at all before reaching the phloem, an hypothesis could be constructed on the supposition that

a gradient of decreasing concentration of virus exists between epidermis and phloem. However this again is open to the objection that so long as the active virus obtained from the epidermis has not been washed away by much less concentrated material from the phloem, the aphid should lose activity comparatively slowly, as it does when fasting.

Watson & Roberts (1939) suggested that 'Either the virus is taken up by the aphids and affected by internal secretions, or (an) inactivating substance is ejected with the saliva and the virus is inactivated in the plants from which it is obtained', (or, presumably, into which it is introduced). There seems no inherent difficulty in postulating that the oral secretions of aphids, which are only produced when the insect is feeding and not when it is fasting, could contain inhibitors, or that these should be specific in their effects. This seems still to be the most plausible and least complicated explanation for the effects observed.

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Note added in proof. Day & Irzykiewicz, 1953 (*Aust. J. biol. Sci.* **6**, 98), using *Myzus persicae* on Chinese cabbage, record uptakes, in the first $\frac{1}{2}$ hr of feeding, of the same order as those we have obtained. From $\frac{1}{2}$ to 6 hr feeding, uptake appeared to be more rapid than in our experiments.

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EXPERIMENTS ON THE POSSIBLE CONTAMINATION OF HONEY WITH SCHRADAN

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The reported low toxicity of schradan to honey-bees has been confirmed. This has led to the consideration of the possibility that this systemic insecticide might be present in nectar and subsequently appear in honey in an unchanged form.

Using radioactive schradan labelled with ^{32}P , it has been shown that spray applications of this insecticide to mustard and borage plants result in the contamination of nectar. A series of nectar samples taken over a 4-week period following spraying showed on radio assay a progressive decrease in total ^{32}P content and also in the amount of schradan present in proportion to the decomposition products. The highest figure recorded for the schradan content of nectar was 21 p.p.m.

Tests on the stability of schradan in contact with the honey stomach of the bee and also in contact with the enzyme invertase, *in vitro*, showed that no appreciable breakdown occurred. Furthermore it was shown that schradan was stable in contact with honey over a period of 2½ months.

It is concluded that this systemic insecticide may appear in an unchanged form in the honey obtained from the nectar of plants which have been sprayed less than 4 weeks previously.

Systemic insecticides such as schradan have been developed to act selectively against hemipterous pests; they depend for their activity on their solubility in water and their translocation within the plant.

Metcalf & March (1949) showed schradan to be completely inactive to bees and house-flies as a contact poison, and as compared with other organo-phosphorus insecticides, to have little inhibitory effect on bee brain cholinesterase. Weaver (1951) reported that in field trials on cotton treated with 1 lb. of schradan per acre there was little or no mortality among bees which were confined on the crop. There was no indication that the nectar was toxic to the bees.

The following experiments are concerned with confirming the reported low toxicity of schradan to bees, investigating the possibility of such a systemic insecticide contaminating nectar and considering whether this can result in schradan appearing in an unchanged form in honey.

TOXICITY OF SCHRADAN TO BEES

The effectiveness of schradan as a stomach poison and its contact effect when applied as an aqueous spray were determined by the methods described by Glynne Jones & Edwards (1952). In addition, bees were kept in contact with a dry deposit of the insecticide for 1 hr. and then caged. Approximately thirty worker bees were

used in each test, and after treatment they were kept at 30° C. and 65% relative humidity. The results for schradan and parathion are given in Table 1; they clearly confirm those obtained by Metcalf & March (1949), and indicate that whilst schradan can be effective as a stomach poison, the contact effect is negligible. Furthermore, it is considered unlikely that a bee will ingest a lethal dose when collecting either contaminated pollen or surface moisture droplets from plant material treated with normal field concentrations.

TABLE 1. *The toxicities of schradan and parathion to bees*

	Schradan, dose/bee (mg.) ($\times 10^{-2}$)	% kill (24 hr.)	Parathion, dose/bee (mg.) ($\times 10^{-5}$)	% kill (24 hr.)		
Stomach poison tests	25	100	70	100		
	20	85	25	85		
	15	64	6	60		
	10	47	3	43		
	8	17	2.5	28		
	5	10	0.5	0		
	Concn. of spray (%)	Wt. of deposit (mg./ sq. cm.) ($\times 10^{-3}$)	% kill (24 hr.)	Concn. of spray (%)	Wt. of deposit (mg./ sq. cm.) ($\times 10^{-5}$)	% kill (24 hr.)
Contact poison tests	1.00	65	35	72	64	100
	0.50	31	25	72	51	82.5
	0.10	6.5	22	60	42	70.0
	0.05	3.1	0	51	36	42.5
				36	25	20.0
				7.2	5	0.0
Dry film tests	1.00	0.06	7	1.00	0.06	100

Mortality in controls varied from 0 to 6%. Approximately thirty bees used at each concentration.

EXPERIMENTS WITH RADIOACTIVE SCHRADAN

The occurrence of schradan with nectar

Frey-Wyssling & Agthe (1950) consider that nectar represents secreted phloem sap, and also show that the dilution of the nectar is in some cases due to the addition of water from xylem vessels which extend into the nectary. Ripper, Greenslade & Hartley (1950) show that schradan sprayed on leaves can be translocated to other parts of the plant, and hence it seemed likely that schradan would appear in the nectar. Experiments using radioactive schradan labelled with ^{32}P were carried out to examine this possibility.

MATERIALS AND METHODS

Preliminary experiment

Radioactive schradan was supplied by Pest Control Ltd. and an aqueous solution was prepared from this containing 0.85 μC . ^{32}P /ml., 0.14% radioinactive schradan,

and a wetter (0.05% teepol). 99.7% of the ^{32}P supplied was stated by Pest Control Ltd. to be in the chemical form of schradan. An air brush gun (aerograph type NSA), operating at an average air pressure of 20–25 cm. Hg, was used for spraying the solution on to the foliage of fourteen white mustard (*Brassica alba*) plants growing in glasshouse soil. Four sprayings over a period of 11 days were applied and six samples of nectar in all were collected. The first application (about 1.7 ml./plant) was given when the plants showed first flower buds and were 8–12 in. high. The final spraying averaged 2.6 ml./plant, by which time the plants had doubled their size. Subsequent growth in this experiment was also very rapid: some of the plants were about 4 ft. high at the beginning of June, and about 6 ft. by the end of that month. Flowering commenced on the day following the first application, and during the remaining sprayings the flowers were carefully enclosed in cellophane bags. Nectar was collected twice a day for 30 days following the first application of insecticide. Six bulk samples were collected by means of a fine capillary tube. The total number of flowers which contributed to each of the samples was recorded for the later samplings. Details of spraying and sampling dates are given in Table 2.

TABLE 2. *Dates of spraying and sampling of white mustard*

Spray no.	Date of spraying	Sampling no.	Period of collection
I	20 May 1952	1	21–21 May 1952
II	24 May 1952	2	25–29 May 1952
III	27 May 1952	3	30–31 May 1952
IV	31 May 1952	4	1–7 June 1952
		5	7–14 June 1952
		6	20–24 June 1952

Radio-assay. Samples 1–3 were very small, and assay was limited to confirmation of the presence of radioactivity and the determination of total activity. The nectar was diluted to 10 ml. and counted in a M6 liquid counter (Veall, 1948). Radioactive decay and any normal counter fluctuations were allowed for by assaying the parent stock solution before and after each nectar solution. After it became clear that ^{32}P was present in the nectar, its chemical form present in sample 4 was determined by following the general procedure recommended by Ripper *et al.* (1950). The nectar was diluted to 25 ml., counted, and the whole of the sample transferred to a 150 ml. R.B. flask. It was made 0.2N with solid NaOH, refluxed for 15 min., cooled rapidly, made 2N with solid NaOH, transferred quantitatively to a separating funnel and partitioned with an equal volume of chloroform. Under these conditions the unchanged schradan partitions almost exclusively in the chloroform phase and the degradation products remain in the aqueous phase (Heath, Lane & Llewellyn, 1952). After separation, the aqueous and chloroform layers were radio assayed, and correction factors applied to the two counts to convert them into the corresponding values which would have been obtained in water. The statistical precision of assay was rather better than 5% and the recovery during partition (based on the initial

count on the nectar solution) was always within the range 90–105%. Sample no. 5 unfortunately was lost.

The data obtained from the assay of the nectar samples are summarized in Table 3. It shows that all those assayed contained ^{32}P and as partition analysis of sample 4 showed undecomposed schradan present it may be inferred that the insecticide was also present in samples 1–3. The volume of sample 4 was 0.20 ml. which, assuming unit density for the nectar, corresponds to a concentration of 21 p.p.m. schradan in this sample. Sample 6 taken 20–24 days after the final application of insecticide to the plants did not contain any undecomposed schradan.

TABLE 3. *Records of schradan content of nectar*

Sample no.	No. of flowers	Wt. of schradan in nectar assuming no breakdown ($\mu\text{g.}$)	% breakdown of schradan	Wt. of undecomposed schradan in analysed nectar ($\mu\text{g.}$)
1	22	0.12	—	—
2	37	0.75	—	—
3	139	1.05	—	—
4	1028	6.9	38	4.3
5	2571	—	—	—
6	1729	2.9	100	Nil

Although there was no indication whatsoever of direct contamination of the nectar in this experiment, the fact that the spraying and sampling periods overlapped might be put forward as a criticism, and the data obtained in this preliminary experiment must be accepted with some reserve.

Final experiment

A second experiment was therefore planned in which spraying was completed 3 days before sampling commenced and all flowers removed after the completion of spraying. Two species of plants, representing different families and hence differing in floral structure, were selected. They were white mustard (*Brassica alba*) and borage (*Borago officinalis*), growing in soil in a glasshouse. In this experiment spraying was completed in 5 days for the mustard plants, and in 6 days for the borage. Growth was very much less vigorous on this occasion—little growth of any of the plants occurred during the time of spraying, and even at the end of the sampling the plants had barely doubled their initial size. During spraying, which was carried out as in the preliminary experiment, the leaves were backed with filter-paper shields which were subsequently acid digested and radio assayed to obtain an estimate of the non-impacting proportion of the spray. Spraying on each occasion was stopped before run-off occurred.

The radioactive schradan was again supplied by Pest Control Ltd. An aqueous stock solution was prepared containing 0.16% radioinactive schradan, 0.75 $\mu\text{C. }^{32}\text{P}/\text{ml.}$, and 0.05% teepol as wetter. In this case 97.8% of the ^{32}P was stated by the firm to be in

the chemical form of schradan. An approximate confirmation of this value was obtained by determining the partition coefficient of the stock solution (made 1N with NaOH), with an equal volume of chloroform. A partition coefficient of 21 was obtained at room temperature (Hartley, Heath, Hulme, Pound & Whittaker 1951).

Details of spraying. White mustard. Nineteen plants sprayed in five applications: 26, 27, 28, 29 and 30 July—94.4 ml. of solution sprayed from gun, of which about 78 ml. reached the foliage.

Borage. Thirteen plants sprayed in six applications: 26, 27, 28, 29, 30 and 31 July—45 ml. of solution sprayed from gun, of which about 38 ml. reached the foliage.

All opened flowers were removed from the plants on 31 July and 1 August 1952 to make quite certain that direct contamination of sampled flowers had not occurred during the spraying. Sampling began on 3 August 1952.

Details of sampling. Less nectar was secreted by the mustard flowers than during the preliminary experiment, and only three bulk samples could be obtained during the flowering period. The borage plants, however, secreted nectar very freely, and seven samples were obtained during the 4 weeks following the completion of spraying.

Radio-assay. All the nectar samples were weighed and made up to volume in aqueous solution—25 ml. for the first samples and smaller volumes for later ones. After counting—to give total activity—partitioning was carried out following the procedure outlined above. Great care was taken in carrying out these partitions to avoid radioactive contamination. Recoveries during partition varied between 90 and 105% in all cases, except for the sample borage 7 where the recovery was only 75%. In view of the low counts involved, counting times of between 30 and 60 min. were general for background and sample counting.

As an illustration of the order of counts involved and of the somewhat lower precision obtained with the later samples, details of the partition of the sample borage 4 are given below (Table 4).

TABLE 4. *Partitioning of the sample borage 4 nectar, diluted to 20 ml. with equal volume of chloroform*

Aqueous (2N alkali) layer	13.28 counts/min.	S.E. \pm 1.05
CHCl ₃ layer	5.45 counts/min.	S.E. \pm 1.16

After applying the necessary corrections for density, etc., these data lead to a value of $67 \pm 10\%$ for the breakdown of schradan in this nectar sample.

The complete data obtained in the final experiment are summarized in Table 5. Schradan was again shown to be present in the nectar from mustard and also in that from borage. The degree of breakdown and hence the actual concentration of schradan in the nectar is related to the date of sampling. No appreciable amount of schradan was present in the nectar of either of the two species after approximately 28 days had elapsed since the last application of the foliage spray.

The significance of small amounts of pollen present in the nectar samples. All the

TABLE 5. *Sampling dates and summary of radio-assay in final experiment*

Nectar sample	Date of sampling (August)	Wt. of sample (g.)	% breakdown of schradan in nectar	Wt. of undecomposed schradan (μ g.)	Concn. of undecomposed schradan in nectar (p.p.m.)
Mustard 1	3-12	0.46	50	2.5	5.5
2	12-20	0.41	77	0.6	1.4
3	21-28	0.20	Assume 90*	0.06 (estimate)	0.3 (estimate)
Borage 1	3-7	1.00	16	2.5	2.5
2	7-11	1.00	20	1.3	1.3
3	11-14	1.11	60	0.6 ₅	0.5 ₅
4	14-18	1.25	67	0.6	0.5
5	18-21	1.09	74	0.3 ₃	0.3
6	21-25	0.60	93	0.05 ₅	0.09 ₉
7	25-28	1.14	95	0.0 ₈	0.0 ₇

* Not partitioned because of low count -8.8 ± 0.7 per min. (in 10 ml.)

nectar samples were slightly contaminated with pollen, and it was therefore thought possible that any radioactivity in the pollen might have an appreciable effect on the total. To test this point 200 anthers from mustard plants previously sprayed with radioactive stock solution were acid digested and a total ^{32}P count carried out on the digest on 26 August 1952. The digest contained $0.76 \mu\text{g.}$ of schradan assuming that no decomposition had occurred. Since the pollen present in 200 anthers would be at least 20 times the amount in the largest nectar sample it is unlikely that the pollen contamination was an important factor in this experiment.

OBSERVATIONS AND EXPERIMENTS ON THE STABILITY OF SCHRADAN WHEN NECTAR CONTAINING THE INSECTICIDE IS PROCESSED INTO HONEY

The foraging bee stores nectar in her honey stomach and, on returning to the hive, the nectar is transferred to a house bee. Whilst it is in the honey stomach of the forager the enzyme invertase, a product of the salivary glands, is added (Oertel, Fieger, Williams & Andrews 1951), and this initiates the inversion of the sugars present in the nectar. The house bees manipulate the nectar with their mouth-parts in such a way so as to spread each drop over a large surface area and this facilitates the evaporation of excess water. The nectar is eventually placed in a wax cell in the comb where further evaporation occurs until the water content is reduced to less than 20%. Finally, the honey so produced is sealed over with a waxen cap.

It was desirable, therefore, to ascertain whether schradan is affected in any way during the inversion of the sugars and also to determine its fate on prolonged storage in honey. Oertel *et al.* (1951) have shown that the inversion of sucrose in the honey stomach is a rapid process (up to 81.4% inversion could occur in $2\frac{1}{2}$ hr.). The following experiment was undertaken to determine if any decomposition of schradan occurred when it was kept with sucrose in the honey stomachs of living bees.

Worker bees were trained to feed from a dish containing a 0.064% solution of radioactive schradan made up in 50% sugar syrup. The bees did not appear to notice that the sugar syrup was adulterated, and after taking the syrup the bees returned to their hives and a few minutes later revisited the dish. This was shown by marking the bees. A number of bees were caught and caged as they were about to leave the dish after feeding. After 3 hr. no unusual symptoms were seen amongst the caged bees, and those released were capable of normal flight. Ten of the remaining bees were killed and their honey stomachs carefully removed. The contents of the honey stomachs were subjected to a partition analysis 3 days later, and the results indicated that less than 3% of the schradan had been decomposed. 56 μ g. of undecomposed schradan was found in the mush.

In a further experiment, the salivary glands of 180 worker bees were roughly dissected out and macerated with 40 ml. of distilled water. This was filtered and to the filtrate 10 ml. of a 0.14% radioactive schradan solution was added. This solution was maintained for 7 days at 30° C., a temperature approximating to that of the hive. The pH of the system after mixing was 4.61. A subsequent partition analysis indicated that the breakdown of the schradan was less than 3.5%.

In order to determine the rate of breakdown (if any) during the storage of schradan in honey, the radioactive material was mixed with two different honeys for varying periods and kept at 30° C. Honey no. 1 was from pure wild white clover (*Trifolium repens*) and it was obtained fresh from a colony before it had been sealed over in the comb with wax. The moisture content was high, 23%, and the pH was 4.53. Honey no. 2 was a granulated sample, obtained from a mixed flora 11 months previously. Its moisture content was 17.8% and pH 4.16. In the first instance two mixtures were made with each honey, one contained 10 g. of the honey mixed with 10 ml. of a 0.14% radioactive schradan solution and the other contained the same amount of honey and schradan but in addition 30 ml. of distilled water. A trace of thymol was added to the dilute solutions to inhibit fermentation. All the solutions were stored in an incubator at 30° C. for 7 days and then subjected to a partition analysis. The results indicated that virtually no breakdown had occurred under any of the storage conditions described.

A further two tests using honey no. 2 over periods of 1 and 2½ months were undertaken and the breakdown figures in both cases were under 5%, possibly much lower.

DISCUSSION

The results obtained in these experiments show clearly that nectar from borage and mustard flowers may contain appreciable concentrations of undecomposed schradan during the few weeks following the application of the insecticide as a foliage spray. The data also show that this concentration is probably highest at some time during the first few days after spraying, falling to very low values after about a month, by which time most, if not all, of the schradan is likely to have decomposed within the

plant tissue. This work has taken no account of schradan concentration in nectar which might well follow from direct spraying of open flowers—direct contamination in such an event would be much greater. We have in fact taken great care to avoid direct contamination of this kind in both experiments, and in the second experiment, with mustard and borage, the nectar was taken from flowers which were either small buds or even unformed when the spraying was carried out. In addition, it is recognized that some of the metabolic products of schradan are responsible for its toxic action, but as these are unstable, this aspect was not considered.

In view of the high concentration (21 p.p.m.) of schradan in nectar found at the fourth sampling in the preliminary experiment (Exp. 1) and the lower value (5.5 p.p.m.) observed in the second experiment, it is of interest to analyse the factors which may be responsible for this difference. The total volume of spray applied per plant was about twice as great in the preliminary experiment as in the second experiment, and again comparing sample no. 4 in Exp. 1 with sample no. 1 in Exp. 2 (mustard) it is seen that the spraying and sampling procedures are not strictly comparable. Collection of sample no. 4 in the preliminary experiment was begun on the day following the completion of spraying (which had taken 11 days to complete), whilst in the second experiment the collection was not started until 3 days after the spraying programme had been completed. In this latter case spraying had occupied 5 days. The rapid fall off in schradan concentration shown in Table 5 indicates that higher concentrations than 5.5 and 2.5 p.p.m. existed just after the completion of spraying.

The slower secretion of nectar observed with mustard in the second experiment might be an indication of the slowing down of translocation within the plant and this in turn could explain the lower schradan concentrations. Climatic differences undoubtedly existed, and also the day length was shorter during the second experiment.

The appearance of translocated schradan in secreted nectar thus having been established, the possibility of its decomposition within the bee or during more prolonged storage as honey within the hive must be considered. The average load of nectar picked up by a foraging bee is about 40 mg. from 20 to 200 flowers. This is stored in the honey stomach, the walls of which are apparently impermeable to dissolved sugar and water molecules (Pasedach-Poevlerlein, 1940). It is therefore unlikely that the schradan would be absorbed into the bee whilst being transported, and tests have shown that schradan remains undecomposed in the presence of invertase and sucrose. Finally there was the question of schradan stability in contact with honey. In no case considered was there any indication of appreciable schradan decomposition. Since it is clear from the results of bio-assay that schradan presents only a very low toxicity to the foraging bee, it appears very probable that schradan sprayed on foliage of plants, the flowers of which are visited by the honey-bee, may well appear in an unchanged form in the honey.

The water content of mustard nectar varies considerably (20–95 %), but an

examination of the literature suggests that for the purposes of this discussion we may adopt a figure of 70% as an average water content in mustard nectar. During the production of honey from nectar, the water content is reduced to about 17% so that 1 g. of nectar from mustard would yield about 0.47 g. honey. On this basis the concentration of schradan would be doubled in honey derived from nectar containing schradan. David, Hartley, Heath & Pound (1951) suggest an upper limit of 3 p.p.m. for permissible schradan concentration in food crops which constitute a minor or seasonal article of diet, or as in the case of hops, one which is extremely diluted before use. The highest concentration of schradan in honey calculated in this way would be 11.7 p.p.m. in the second experiment and 44.5 p.p.m. in the preliminary experiment. The lower and probably more reliable value (11.7 p.p.m.) corresponds to 5.3 mg. undecomposed schradan per lb. of honey, and since a lb. of honey may be consumed within a few days by one person, this aspect of food contamination must be given careful consideration. These values may by no means present maximum values and might be different in other crops.

At present, however, there is no evidence that schradan is used in Great Britain on crops about to flower which are visited by honey-bees. At the same time it is possible that subsidiary weed crops may inadvertently be sprayed. The main object of this paper, however, is to point out that contamination of nectar and subsequently of honey may occur. Further work with different plants and different spray concentrations on this and a larger scale are necessary for a fuller appreciation of the precise extent of hazard involved.

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FACTORS AFFECTING THE PRODUCTION OF LOCAL LESIONS BY PLANT VIRUSES

II. SOME EFFECTS OF LIGHT, DARKNESS AND TEMPERATURE

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(With 3 Text-figures)

Beans inoculated with tobacco necrosis virus were kept in the dark at different temperatures for 1 hr. before and 1 hr. after inoculation; in this experiment the number of lesions increased with temperature over the range 55–82° F.

The effect of 30 min. periods of darkness before or after inoculation depended on the time of day, the number of local lesions usually being decreased. Prolonging the night period before inoculation sometimes increased the number of lesions.

Light appeared to be more important than temperature in controlling the daily variation in susceptibility. However, in a test over a 30 hr. period this variation continued even when plants were placed under constant conditions before and after inoculation.

When plants that had been kept in the dark were exposed to light of about 800 f.c. intensity for 1 min. immediately before inoculation the number of local lesions was doubled.

Matthews (1953) showed that in an uncontrolled glasshouse the number of local lesions produced by a tobacco necrosis virus in bean varied systematically with the time of day, being greatest when inoculations were made in the afternoon and smallest towards dawn. The present paper deals with some effects of altered conditions of light, darkness and temperature on this 'natural' daily cycle of susceptibility.

MATERIALS AND METHODS

Experiments were made between May and August 1952 on bean plants, var. Sydney Wonder, grown two per 4 in. pot, and used when the primary pair of leaves were well expanded. Growing points were removed before an experiment began. Leaves were lightly dusted with fine carborundum before inoculation, and not washed afterwards. A bulk dilution of bean sap containing tobacco necrosis virus (Fry, 1952) was kept at 4° C. and aliquots were taken for each inoculation time.

In a preliminary trial half of every leaf was inoculated at a base time (Matthews, 1953) before the experimental period. It was found, however, that number of lesions on the standard half leaves for the 'light' treatments was significantly higher (at 5% level) than those on the 'dark' treatments.

The design was therefore modified as follows: Uniform plants in groups of eight pots of two plants were used for each 'treatment'. Any variation in size was

distributed as evenly as possible between treatments, the no. 1 pots for each treatment containing the biggest pairs of plants and so on. Both before and after the experimental period plants were arranged by pot numbers in blocks in the glasshouse. Thus, in the analysis of variance, block variance accounted for effects of both plant size and of position.

Analysis of variance was carried out on the logarithms of the total number of local lesions per pot. In presenting results $\log M$ is plotted against time of inoculation where

$$\log M = \frac{\sum \log (x + 1)}{n},$$

where x = no. of local lesions per pot, n = no. of pots.

Plants for 'dark' treatments were placed in groups of eight pots under boxes on the glasshouse bench. For 'shade' treatments plants were placed under a scrim-covered frame. Except where noted otherwise, plants were placed by numbers in blocks. Inoculation of a set of sixteen plants took 5–8 min. Sets to be inoculated together were always inoculated in the same order. In presenting results the difference from the nominal time has been ignored.

Light was measured with a Weston exposure meter by reflexion from a sheet of white filter-paper placed horizontally at the level of the leaves.

*Effects of altered conditions on the numbers of local lesions produced
by inoculation at different times of day*

Exp. 1. Effects of darkness and shade. Eight sets of eight pots of two plants were placed at approximately 4 p.m. under each of the following conditions: in light, shade or darkness in a glasshouse with variable temperature; in light or darkness in a glasshouse with constant temperature. Daylight ended about 5.35 p.m. and the first inoculation time was 4 a.m. After each inoculation plants were placed by pot numbers in trays in the respective glasshouses. After the last inoculations all treatments were placed in the constant temperature glasshouse at $70 \pm 2^\circ$ F. In the variable temperature house temperature was 56° F. at 6 a.m., 73° F. from 12 to 4 p.m. and 58° F. by 10 p.m. Temperature in the 'dark' boxes was close to that in the open glasshouse. Light throughout the day was almost identical in the constant temperature and in the variable temperature houses. In the shade the light was about one-tenth that in full light.

Local lesions were counted on the third and fourth days after inoculation. Results are summarized in Fig. 1.

In all groups lesion numbers rose during the day to a maximum at 5 p.m. Darkness before inoculation increased the number of local lesions. Shading caused a smaller increase. For all inoculation times taken together, darkness at 70° produced significantly more lesions (at 5% P) than darkness at variable temperature (56 – 73° F.). However, at 2 and 5 p.m. the latter produced more lesions. In the light the curves were very similar for both variable and constant temperature. For

these two treatments there was about a tenfold increase in numbers of lesions produced for inoculation times between 6 a.m. and 5 p.m. The fewest lesions produced by a set of sixteen plants was 171 while the most was 3258.

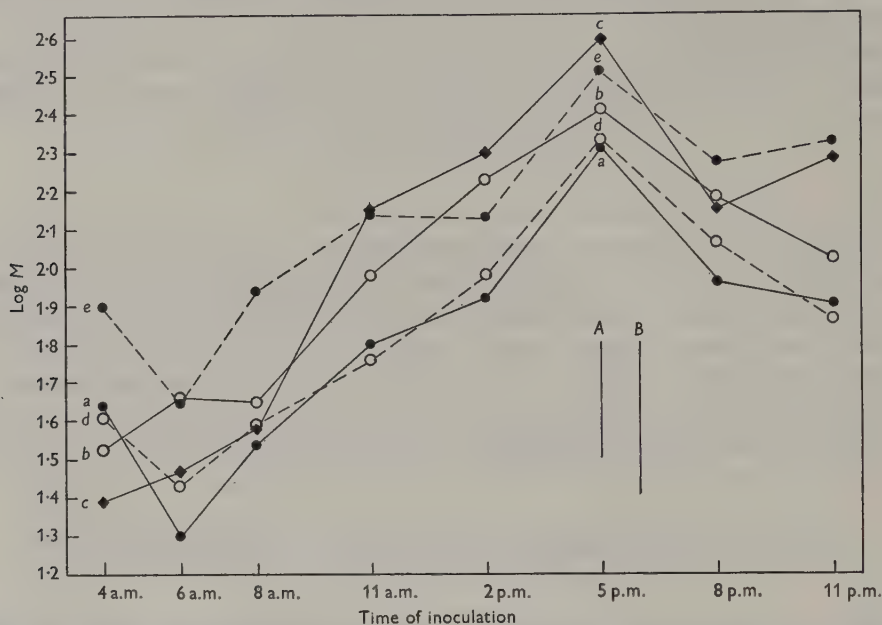


Fig. 1. The effect of light, shade and dark before inoculation on the number of local lesions produced by tobacco necrosis virus in beans. Curve *a*=in light, variable temperature; curve *b*=in shade, variable temperature; curve *c*=in dark, variable temperature; curve *d*=in light, constant temperature; curve *e*=in dark, constant temperature. Lines represent differences required for significance as follows: *A* between any two points at 5%; *B* between any two points at 1%.

Exp. 2. Effect of dark and light before inoculation over a 2-day period. Eighteen groups of eight pots of bean plants were placed as follows between 4.00 and 4.30 p.m.:

- (i) in a glasshouse with uncontrolled temperature and light;
- (ii) in a glasshouse in the dark at $70 \pm 2^\circ \text{F.}$;
- (iii) in a room under artificial light at $68 \pm 0.5^\circ \text{F.}$ (continuous light was supplied by fluorescent tubes giving 13–25 f.c. at leaf level).

The first inoculation time was 7 p.m. Immediately after inoculation all plants were arranged by pot numbers in trays in the constant temperature glasshouse used for (ii). The period of darkness was from about 5.45 p.m. to 7 a.m. On both days in treatment (i) temperature was between 54 and 56°F. from 11 p.m. to 7 a.m., rising steadily to a maximum of 76 – 78°F. about 1 p.m.

Fig. 2 summarizes lesion counts made on the 5th and 6th days after inoculation.

For plants under uncontrolled glasshouse conditions the maximum was at 10 a.m. on each day.

For plants given continuous artificial illumination before inoculation there were maxima at 10 a.m., 10 p.m., 10 a.m. and a rise to 10 p.m. on the second day. For plants in continuous darkness before inoculation there were maxima at 10 a.m.

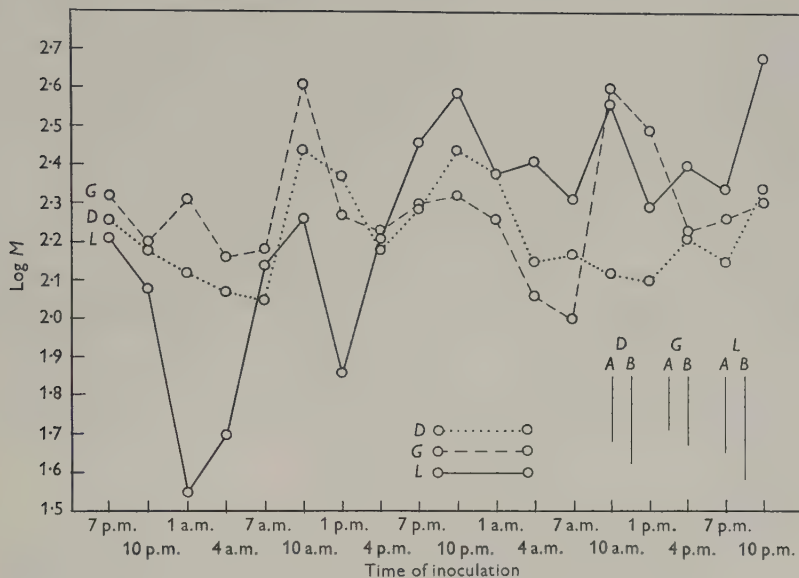


Fig. 2. Effect of continuous light or dark before inoculation on the number of local lesions produced by tobacco necrosis virus in beans. Curve *L*=continuous light before inoculation; curve *D*=continuous dark before inoculation; curve *G*=uncontrolled glasshouse conditions. Lines represent differences required for significance within each curve as follows: *A* between any two points at 5%; *B* between any two points at 1%.

and 10 p.m. on the first day but no maximum during the second day. For most times of inoculation plants kept in continuous darkness before inoculation produced fewer lesions than plants in the uncontrolled glasshouse.

In this experiment the standard errors of $\log(x+1)$ were not homogeneous for the three treatments. For this reason significant differences for each curve alone have been given. The standard errors of $\log(x+1)$ were: glasshouse, 0.140; dark before, 0.174; light before, 0.205. Difference required for significance: at 5% $P=0.036$; at 1% $P=0.047$.

Exp. 3. Effect of continuous light and dark before and after inoculation. Between 3.40 and 4.30 p.m. forty sets of eight pots of bean plants were placed in a room at $69 \pm 0.5^\circ \text{F.}$ and $70 \pm 5\% \text{ R.H.}$ The light source was as used in Exp. 2. Ten sets of plants were given one of the following treatments: (i) constant illumination before and after inoculation ($L \rightarrow L$); (ii) darkness before and after inoculation ($D \rightarrow D$);

(iii) light before, and dark after inoculation ($L \rightarrow D$); (iv) dark before and light after inoculation ($D \rightarrow L$).

The first inoculation was at 7 p.m.: 36 hr. later local lesions were well developed on the first inoculated plants in the $L \rightarrow L$ and the $D \rightarrow L$ treatments. Lesions

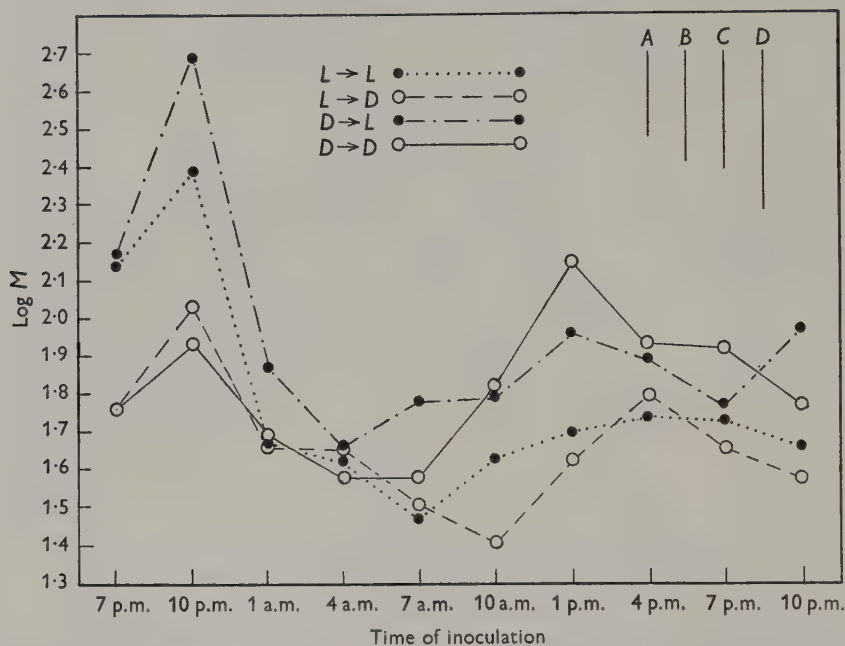


Fig. 3. Effect of continuous light and dark, at constant temperature before and after inoculation on the number of local lesions produced by tobacco necrosis virus in beans. $L \rightarrow L$, light before and after inoculation; $L \rightarrow D$, light before, dark after inoculation; $D \rightarrow L$, dark before, light after inoculation; $D \rightarrow D$, dark before and after inoculation. Lines represent differences required for significance as follows: A between any two points in same curve at 5%; B between any two points in same curve at 1%; C between any two points at 5%; D between any two points at 1%.

appeared some hours later in the $D \rightarrow D$ and $L \rightarrow D$ plants. Lesions on all groups were counted between 60 and 68 hr. after inoculation, and plants were removed to the glasshouse.

At this time the $D \rightarrow D$ treatments had much paler leaves than the $L \rightarrow L$ plants. The latest inoculated plants showed few local lesions except those in $L \rightarrow L$. The following series showed an increase on a recount made 3 days later, and this second count was used: $D \rightarrow D$, all counts from 7 a.m. to 10 p.m.; $L \rightarrow D$, 4, 7 and 10 p.m.; $D \rightarrow L$, 7 and 10 p.m. Results are summarized in Fig. 3.

For all treatments there was a rise to a maximum at 10 p.m., 6 hr. after plants had been placed under the differential conditions. This rise was greatest for the

$D \rightarrow L$ and $L \rightarrow L$ treatments. For all treatments there was a fall after 10 p.m. and a significant rise the following day. This rise was greatest for the $D \rightarrow D$ and $L \rightarrow D$ treatments and least for $L \rightarrow L$.

Some effects of altered conditions for short periods on the number of local lesions produced

Exp. 4. Exposure to various temperatures in the dark for about 1 hr. before and 1 hr. after inoculation. Groups of nine pots of two bean plants were placed in dark cabinets at different temperatures at approximately 2.20 p.m., and inoculated between 3.20 and 4.05 p.m., beginning and ending with a control group in the glasshouse at 70° F. No plant was out of its cabinet for more than 1 min. for inoculation. Before and after the experimental period plants were kept in a glasshouse at $70 \pm 2^\circ$ F. Results of local lesion counts are summarized in Table 1.

TABLE 1. *Effect of various temperatures in the dark for 1 hr. before and after inoculation on number of local lesions produced by tobacco necrosis virus in beans*

Temperature setting of cabinet (° F.)	Temperature in cabinet at leaf level. Mean of two readings. (° F.)	Log M^*	Mean no. of local lesions per leaf
55	55	1.53	8
63	65	1.56	9
70	69	1.74	14
80	75	1.77	17
90	82	2.00	26
Control A	70° F. } in	2.12	40
Control B	70° F. } light	2.27	45

* Difference required for significance; at 5% P 0.19; at 1% P 0.25.

Plants placed in the dark for 1 hr. before and after inoculation showed fewer local lesions at all temperatures than did control plants in the glasshouse at 70° F. There was a significant trend of increasing number of local lesions with increasing temperature.

Exp. 5. Effects of 5-45 min. periods in the dark before and after inoculation. Groups of eight pots of two bean plants were placed in the dark for 5, 15, or 45 min. before or after inoculation. Each set of plants had a control set, and one pot of test plants and one pot of control plants were inoculated alternately. The experiment was done in a glasshouse at $70 \pm 2^\circ$ F., between 1.05 and 2.15 p.m. Results are summarized in Table 2.

Plants placed in the dark for 15 or 45 min. before inoculation showed significantly fewer local lesions than undarkened controls. Treatments after inoculation had no significant effects.

Exp. 6. Effect of 30 min. darkness before or after inoculation at different times of day. Groups of eight pots of two plants were placed in the dark for 30 min. before or

after inoculation, each set of eight treated plants having a control set as in Exp. 5. Sets of plants were inoculated at 8, 11 a.m., 2 and 5 p.m. in a glasshouse at $70 \pm 2^\circ \text{F}$. Results are summarized in Table 3.

TABLE 2. *Effects of 5-45 min. periods in the dark before and after inoculation, on number of local lesions produced by tobacco necrosis virus in beans*

Dark treatment	Log <i>M</i> for		Mean no. of local lesions per leaf	
	Control	Treatment	Control	Treatment
Minutes before				
5	2.26	2.10	48	33
15	2.13	1.86	34	19
45	2.25	1.86	49	22
Minutes after				
5	2.31	2.34	60	58
15	2.40	2.55	70	101
45	2.23	2.28	46	71

Difference for significance between values of log *M* for any treatment and its control; at 5% $P=0.20$, at 1% $P=0.27$.

TABLE 3. *Effect of 30 min. darkness before or after inoculation, at various times of day, on the number of local lesions produced by tobacco necrosis virus in beans*

30 min. dark period	Time of inoculation	Log <i>R</i> *	Mean no. of local lesions per leaf	
			Control	Treatment
Before inoculation	8 a.m.	-0.19	29	18
	11 a.m.	0.09	42	52
	2 p.m.	0.13	43	53
	5 p.m.	0.14	49	63
After inoculation	8 a.m.	-0.42	23	8
	11 a.m.	-0.32	46	23
	2 p.m.	-0.21	42	28
	5 p.m.	-0.11	46	41

$$* \text{Log } R = \frac{\sum \log(t+1) - \sum \log(c+1)}{8},$$

where *t*=no. local lesions per pot (two plants) for treated plants; *c*=no. local lesions per pot (two plants) for control plants.

Differences required for significance:

	at 5% <i>P</i>	at 1% <i>P</i>
Between any two values of <i>R</i>	0.34	0.45
Between two values of <i>R</i> at the same time	0.29	0.39
For any value of <i>R</i> from zero	0.24	0.32

The mean values of *R* for the pre-inoculation treatments was 0.004 and for the post-inoculation treatments -0.26, the differences required for significance being 0.12 at 5% *P* and 0.18 at 1% *P*. For the data of the pre- and post-inoculation treatments considered in one analysis, the correlation coefficient $r=0.916$ ($r=0.875$). The regression coefficient of log *R* on time in hours = 0.034/hr.

Thus dark treatment after inoculation over all times reduced the number of local lesions more than did treatment before inoculation. The effect of treatment depended on time of day of inoculation. The numbers of lesions produced by treated plants relative to controls increased from 8 a.m. to 5 p.m.

Exp. 7. Effect of short periods of light on number of local lesions produced by plants kept in the dark. Four sets of eight pots each containing two bean plants were placed at 4.30 p.m. in the dark at $69 \pm 0.5^\circ \text{F}$. Next day, between 10.50 and 11.30 a.m., one set of eight plants was exposed for 5 min. to light of about 200 f.c. from a single 500 W. bulb. Plants were exposed, and inoculated in two sets of four pots. Exposed plants, along with controls, were inoculated immediately after treatment. A second set of eight plants was exposed to light of about 800 f.c. from four 500 W. bulbs for 1 min. In spite of a system of baffles and fans the temperature rose from 70–72 to 75–77° F. at plant level for both the 5 min. and the 1 min. exposures. After treatment plants were placed again in the dark at $69 \pm 0.5^\circ \text{F}$. Results are summarized in Table 4.

TABLE 4. *Effect of short exposure to light on the production of local lesions by tobacco necrosis virus in beans*

Treatment	Log <i>M</i> *		Mean no. of local lesions per leaf	
	Control	Treatment	Control	Treatment
200 f.c. for 5 min.	1.57	1.65	15	17
800 f.c. for 1 min.	1.51	1.87	13	28

* Difference required for significance: at 5% $P=0.13$, at 1% $P=0.18$.

Exposure to 800 f.c. for 1 min. significantly increased the number of local lesions, while exposure to 200 f.c. for 5 min. did not.

DISCUSSION

Bawden & Roberts (1948), using tobacco necrosis virus in beans, showed that keeping plants in the dark for 24 hr. before inoculation increased the number of local lesions produced. Inoculations were made between 3 and 5 p.m. and no details were given of results for periods of darkness shorter than 24 hr.

The results of Exp. 1 at the time 25 hr. after plants had been placed under differential conditions confirm the results of Bawden & Roberts. This result was not confirmed in Exp. 2, where, over most of the period tested, plants kept in the dark before inoculation produced fewer local lesions than those under normal glass-house conditions. Temperature differences between the glasshouse and dark treatments in this experiment may account for fewer lesions being produced by the dark treatments. An alternative explanation is suggested by the work of Hitchborn (1952). He has found that the response of beans to increased dark periods shows a seasonal variation and therefore presumably depends on the conditions under which plants

are raised. Under winter light conditions he found dark periods of 1-4 days before inoculation decreased, while under summer conditions darkness increased the number of lesions produced by a tobacco necrosis virus in beans. Kassanis (1952) found that keeping plants at 36° C. for some time before inoculation greatly increased the number of local lesions produced by several viruses, while treatment at 36° C. after inoculation reduced the number of local lesions with two viruses and completely prevented lesion formation with three others including a tobacco necrosis virus. In the present work it was found (Exp. 4) that increasing temperature for 1 hr. before and 1 hr. after inoculation increased the number of local lesions produced. No detailed comparison of these results can be made, since Kassanis used much longer times of treatment, usually one day or more.

The results of Exp. 1 suggest that temperature may play a relatively small part compared with light in causing the daily fluctuation in susceptibility of plants. In Exps. 1 and 2 the effects of treatments before inoculation are almost certainly partially obscured by the varying periods of light and darkness which the plants received after inoculation. Such effects may account for the 12 hr. periodicity found for plants receiving continuous light before inoculation.

In Exp. 3 plants at constant temperature and in continuous darkness before and after inoculation showed a maximum of susceptibility at 1 p.m. This suggests that the daily fluctuation in susceptibility may continue for a time after the varying conditions have been eliminated. Similar phenomena have been found for certain nastic movements in plants (Stiles, 1950).

With plants in continuous light before and after inoculation the rise in susceptibility during the day was less. If, as seems likely, light is the main cause of the daily fluctuations in susceptibility, its continuance at a low level may be more effective than darkness in lessening the daily change in susceptibility.

Although there is no doubt that artificially extending the night period before inoculation can increase the number of local lesions, placing plants in the dark for short periods before or after inoculation during the day can reduce the number.

The daily cycle of susceptibility will affect the results both of experiments carried out at different times on the same day, and at the same time on different days. In Exp. 6 the effect of 30 min. darkness before or after inoculation was shown to depend on the time of day at which the treatment was applied. In a number of experiments with tobacco necrosis virus in beans the time of maximum susceptibility has been found to range from 10 a.m. to 5 p.m. Therefore, in experiments carried out on different days plants may be in a different state at the same time of day. Such effects may account for the reverse results obtained in Exps. 5 and 6 for plants inoculated about 2 p.m.

In Exp. 7, exposure of plants, previously held in the dark, to light of about 800 f.c. for 1 min. immediately before inoculation doubled the numbers of local lesions. This effect was probably due to the light and not to the rise in temperature, as a similar rise in temperature without any effect on numbers of local lesions occurred

with plants exposed to 200 f.c. for 5 min. This result suggests the possibility that the susceptibility of cells may be increased by some early product of photosynthesis.

I wish to thank Mr G. C. Ward, Applied Mathematics Laboratory, N.Z. Department of Scientific and Industrial Research, for the statistical analyses.

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THE MUTUAL EFFECT OF RYEGRASS AND CLOVER WHEN GROWN TOGETHER

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Italian ryegrass and a late-flowering red clover were grown together, with abundance of water and nutrients for both. It was found that even a small number of ryegrass plants reduced the growth of clover by 30 %. This effect varied very little with increasing density of the clover crop.

The presence of clover reduced the ryegrass crop by an amount diminishing as the density of the ryegrass was increased. In a sparse crop of ryegrass, clover reduced the growth of the grass considerably more than did barley under comparable conditions.

There is no evidence of any specific effect of the roots of one plant on the other. When ample nitrogen is available the clover tends to take some that would otherwise be available for the grass and does not provide the grass with additional nitrogen.

INTRODUCTION

Mann & Barnes (1945, 1947, 1949, 1950, 1952) have described the effect of various weeds on barley and that of barley on the weeds, when they are grown together. But the mutual influence of agricultural plants is not limited to the effect of weeds. In some cases, notably in the sowing down of leys, a mixture of plants is deliberately sown, each of which is expected to make its contribution to the value of the crop obtained. It seemed therefore of interest to apply the methods which we have used in the study of weeds to the case of mixtures of grass and clover. For this purpose we chose a mixture of Italian ryegrass and broad red clover.

As described previously the experiments were conducted in conditions where the root space was constant but limited, where there was always sufficient water supply, though the soil was well aerated, and where the phosphate and potash application was enough for both the ryegrass and the clover. Nitrogen was added in the form of ammonium sulphate sufficient to maintain the ryegrass in a green and healthy condition but not enough to damage the growth of the clover. Both single and mixed crops were grown in earthenware pots 28 cm. in diameter and 25.5 cm. deep, furnished with an upturned outlet near the bottom which enabled watering to be done without danger of loss and yet secured good aeration of the soil. The bottom of the pots was covered with coarse gravel to above the outlet. Each pot was then filled with 16.3 kg. of soil taken from one of the fields at Woburn where both ryegrass and clover grow well. This gave about 20 cm. depth of soil and a volume of 12.3 or 14.2 l., including the gravel.

The first question to consider is how far each of the crops when grown alone is affected by the number of plants of its own kind per unit volume of soil.

Italian ryegrass. In each of the pots already described ryegrass plants varying in number from 1 to 8 were grown. All the treatments were in duplicate. Each pot received nitrogen in the form of ammonium sulphate at the first sign of slight yellowing of the growth. The amount added (nitrogen per pot) to the ryegrass sown on 1 April was: 24 May, 0.25 g.; 11 June, 0.25 g.; 22 June, 0.25 g. After the first crop of ryegrass was cut a further addition of 0.25 g. nitrogen per pot was made on 19 August. These additions enabled the ryegrass to grow satisfactorily without dependence on the clover for nitrogen. The ryegrass was cut on 22 July and again on 28–29 September. Table 1 shows the results for this crop grown alone.

TABLE 1. *Results of competition between ryegrass plants grown alone*

No. of ryegrass plants per pot	Root space per plant (l.)	Max. no. of shoots (1st crop)		Air-dry weights of produce (g.)			Stubbles (g.)
		Per pot	Per plant	1st crop	2nd crop	Total	
1	14.2	79	79	48.0	23.7	71.7	23.2
2	7.1	77	39	45.9	24.0	69.9	29.4
4	3.5	116	29	49.9	22.8	72.7	28.2
6	2.4	151	25	47.3	28.2	75.5	29.1
8	1.8	177	22	42.3	23.5	65.8	37.1

The yield, both at the first and the second cutting, is remarkably constant, whatever be the number of the plants. Apparently, when the ryegrass is supplied with sufficient nitrogen and with water, the growth of each plant is almost proportional to the root space available. The total yield does not decrease seriously until there are at least eight plants per pot or until the root space is less than 1.8 l. per plant, and, on the other hand, the yield from a single plant is almost equal to that of a larger number of plants with the same total root space.

Clover. When clover is grown alone (Montgomery late-flowering red clover was used) the results obtained are a little different. Table 2 shows the yields obtained in the first and second crops. With clover, therefore, the yield becomes greater as the number of plants per unit volume of soil is increased up to at least six plants per pot with a soil volume of 2.4 l. per plant. The mean proportion of the roots to the first crop, the stubbles, and the roots combined is 13.0%.

TABLE 2. *Results of competition between clover plants grown alone*

No. of clover plants per pot	Root space per plant (l.)	Yield of air-dry produce (g.)				Wt. air-dry clover roots 1948 and 1949 mean (g.)
		1st crop	2nd crop	Stubbles	Total	
1	14.2	71.2	30.7*	4.5	106.4	11.1
2	7.1	73.7	18.2	7.8	99.7	11.1
4	3.5	103.9	26.2	8.7	138.8	16.5
6	2.4	108.3	38.5	8.0	154.8	18.1
8	1.8	107.8	39.8	8.1	155.7	18.0

* This figure is from a single pot as the duplicate was lost.

ASSOCIATION OF ITALIAN RYEGRASS AND CLOVER

When Italian ryegrass and clover are grown in association with sufficient nitrogenous fertilizer to secure a green and healthy crop of ryegrass, competition arises between the plants both for space and for nitrogen. Table 3 shows the yields obtained when

TABLE 3. *Yield of ryegrass and clover, with varying density of ryegrass*

No. of plants per pot		Max. no. of ryegrass shoots per plant	Yield of ryegrass (air-dry) (g.)				Yield of clover (air-dry) (g.)				Total dry matter (g.)
Ryegrass	Clover		1st crop	2nd crop	Stubbles	Total	1st crop	2nd crop	Stubbles	Total	
0	6	—	—	—	—	—	108.3	38.5	8.0	154.8	154.8
1	6	31	16.3	18.6	11.3	46.2	85.7	16.6	5.2	107.5	153.7
1	0	79	48.0	23.7	23.2	94.9	—	—	—	—	—
2	6	21	20.7	19.1	12.8	52.6	78.7	18.7	5.8	103.2	155.8
2	0	39	45.9	24.0	29.4	99.3	—	—	—	—	—
4	6	20	30.9	19.6	22.4	72.9	68.1	13.7	3.3	85.1	158.0
4	0	29	49.9	22.8	28.2	100.9	—	—	—	—	—
6	6	18	34.6	22.0	18.3	74.9	62.3	13.9	2.5	78.7	153.6
6	0	25	47.3	28.2	29.1	104.6	—	—	—	—	—
8	6	16	37.3	20.7	16.4	74.4	53.2	17.8	3.9	74.9	149.3
8	0	22	42.3	23.5	37.1	102.9	—	—	—	—	—

the density of the ryegrass was varied from a small to a large proportion of the amount of clover. The density of the clover was slightly greater than is necessary to give a full crop if no other plant is present. It is therefore clear that the ryegrass is growing in a space which would be already fully occupied by clover if the latter were grown alone.

The total weight of air-dry material above ground is almost identical in all cases where clover is grown with ryegrass, and in no case has the presence of the clover improved the yield of the ryegrass. The yield of ryegrass has been reduced by the presence of the full quota of clover by an amount which depends on the proportion of the former to the latter, but when four or more plants of ryegrass occur with six plants of clover in the limited space (14.2 l.) provided, the loss in yield of the ryegrass is the same. As regards the effect of the ryegrass on a full plant of clover, there is always a reduction in the clover yield which increases with the number of ryegrass plants present.

The effect of even a small proportion of ryegrass on the growth of the clover is considerable, though it is substantially less than we have found under comparable conditions with barley (Mann & Barnes, 1952). The influence of the clover in reducing the growth of the ryegrass is substantially greater than we found with barley. Taking all densities of the barley or ryegrass when mixed with a full crop of clover we found the following:

	Percentage reduction in barley or grass	Percentage reduction in clover
With barley	14	58
With ryegrass	36	42

Table 4 illustrates the effect of increasing from 0 to 8 the number of clover plants on the development and yield of two plants per pot of Italian ryegrass.

The presence of clover never increases the yield of a sparsely planted crop of ryegrass, nor, when the number of clover plants is as great or greater than those of ryegrass, is there any further reduction in ryegrass yield. There is no sign that the clover can smother the ryegrass even when it is present in far greater amount than the latter. But, as we found with barley and clover, the presence of even a small amount of ryegrass makes a great difference to the growth of clover.

TABLE 4. *Yield of ryegrass and clover, with various densities of clover*

No. of plants per pot		Max. no. of ryegrass shoots	Yield of ryegrass (g.)				Yield of clover (g.)			
Ryegrass	Clover		1st crop	2nd crop	Stubbles	Total	1st crop	2nd crop	Stubbles	Total
2	0	77	45.9	24.0	29.4	99.3	—	—	—	—
2	1	57	42.6	25.7	19.9	88.2	35.8	6.5	1.5	43.8
0	1	—	—	—	—	—	71.2	30.7	4.5	106.4
2	2	51	34.5	25.2	18.6	78.3	55.1	10.3	3.1	68.5
0	2	—	—	—	—	—	73.7	18.2	7.8	99.7
2	4	64	25.4	18.5	12.3	56.2	67.5	18.8	5.1	91.4
0	4	—	—	—	—	—	103.9	26.2	8.7	138.8
2	6	44	22.7	14.5	11.9	49.1	78.8	29.2	6.6	114.6
0	6	—	—	—	—	—	108.3	38.5	8.0	154.8
2	8	46	26.4	19.6	14.3	60.3	70.9	23.5	5.2	99.6
0	8	—	—	—	—	—	107.8	39.8	8.1	155.7

The clover has, obviously, reduced the total amount of growth of the ryegrass both when the proportion is small and when it is as great as eight plants of clover to two of ryegrass. When the number of clover plants is increased from two to eight, the reduction in the yield of two ryegrass plants, whether for the first crop only or for the total produce including the stubble, is between 23 and 23.5%. This is much greater than the corresponding reduction of barley yield by clover. The effect of ryegrass on the growth of clover, even when the ryegrass is only a sparse plant, is considerable, but does not vary markedly when the amount of clover in relation to the ryegrass is increased. Further, an increase in the number of clover plants from two to eight leads to an increase in yield of 56% in the absence of ryegrass, and of 45.5% in its presence. This would suggest that the effect of the ryegrass varies very little and affects the capacity of the soil to grow clover to an almost similar extent whether the clover crop be thick or thin.

Table 5 shows the effect of increasing congestion in the space available when the same or another species is used for the purpose of increasing the congestion.

TABLE 5. *Comparative reduction in yield of ryegrass and clover due to increase in the number of ryegrass or clover plants*

	Percentage reduction due to increased ryegrass	Percentage reduction due to increased clover
A. Reduction in total produce of 2 ryegrass plants		
(1) With 2 additional ryegrass or 2 clover plants per pot	49.1	21.1
(2) With 4 additional ryegrass or 4 clover plants per pot	64.8	43.4
(3) With 6 additional ryegrass or 6 clover plants per pot	74.2	50.6
B. Reduction in produce of 2, 4, or 6 clover plants		
(1) 2 clover with 2 ryegrass or 2 additional clover plants per pot	31.3	30.3
(2) 4 clover with 2 ryegrass or 2 additional clover plants per pot	34.1	25.6
(3) 6 clover with 2 ryegrass or 2 additional clover plants per pot	25.9	24.5

The effect of increasing the congestion of ryegrass plants on the productivity per plant is very similar to that found with barley, but increasing the clover has a much greater effect on the growth of the ryegrass than of barley (cf. Mann & Barnes, 1952). The effect on clover of additional ryegrass is almost equal to that produced by increasing the congestion of the clover itself.

DISTRIBUTION OF NITROGEN BETWEEN RYEGRASS AND CLOVER

When ryegrass and clover are grown alone or together under the conditions already described, the percentage of nitrogen in the produce is as shown in Table 6. The presence of the clover seems to increase slightly the concentration of nitrogen in the ryegrass both in the first and second crops. No similar increase was found in the clover crop when the latter was mixed with ryegrass.

TABLE 6. *Nitrogen in ryegrass or clover grown alone or together*

	Nitrogen percentage in dry matter			
	Ryegrass grown alone	Ryegrass grown with clover	Clover grown alone	Clover grown with ryegrass
1st crop	1.46	2.08	—	—
2nd crop	1.24	1.59	—	—
Roots	0.67	—	2.50	—
Clover above ground				
1st crop	—	—	2.61	2.67
2nd crop	—	—	3.65	3.31

In our experiments, as stated above, a total of 1 g. of ammonium sulphate was added to each of the pots, and the content of nitrogen in the whole crop, including the roots, was as shown in Table 7. As would be expected, the clover has gained

TABLE 7. *Total nitrogen in whole crops of ryegrass and clover and of mixtures*

	Mg. per pot
Ryegrass grown alone	1270
Clover grown alone	3510
Ryegrass (2 plants) with a mean number of 4.2 plants of clover	3400
Ryegrass (4.2 plants mean) with 6 plants of clover	3284

TABLE 8. *Nitrogen in ryegrass in presence and absence of clover*

No. of plants		Total nitrogen in ryegrass crop per pot (mg.)	Change with clover	
Ryegrass	Clover		mg.	%
2	0	858	—	—
2	1	876	+18	+2.1
2	2	938	+80	+9.3
2	4	909	+51	+5.9
2	6	714	-144	-16.8
2	8	780	-78	-9.1
Mean		843	-15	-1.7

nitrogen from other sources than the nitrogenous fertilizer added. The effect on the nitrogen content of the ryegrass is shown in Table 8. Though the results are not consistent it would appear that the ability of ryegrass to obtain nitrogen has not been appreciably affected by the presence of the clover, nor, certainly, has the presence of the clover increased the nitrogen uptake of the ryegrass. It seems quite clear that, given adequate nitrogen, the ryegrass does not take nitrogen either from the clover or from residues that the latter provides.

Whatever the thickness of planting of the ryegrass the absorption of nitrogen is remarkably constant, but in admixture with clover, when the latter is present in large amount, it cannot and does not secure as much nitrogen as when grown alone unless it is in large proportion relatively to the clover (Table 9).

TABLE 9. *Nitrogen in a variable ryegrass crop in presence of clover*

No. of plants		Total nitrogen in ryegrass above ground		
Ryegrass	Clover	Grown alone (mg.)	Grown with clover (mg.)	Change with clover (%)
1	6	874	522	-40.3
2	6	858	715	-16.7
4	6	834	857	+2.8
6	6	872	840	-3.7
8	6	819	874	+6.7

It should be noticed that in all the cases now under review there was an ample supply of nitrogen for the ryegrass independent of anything which the clover might provide. Under these circumstances the clover acts merely as a competitor with the ryegrass. What would happen if the ryegrass was grown with a deficiency of nitrogen has not been considered, and there is always the possibility that under these conditions the clover accompanying the ryegrass might supply the nitrogen deficiency either from decaying portions of its roots or from its root exudations. To test whether this is so would demand a new series of experiments.

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STUDIES ON THE EFFECT OF ASSOCIATED SOIL MICRO-FLORA ON *FUSARIUM UDUM* BUTL., THE FUNGUS CAUSING WILT OF PIGEON-PEA (*CAJANUS CAJAN* (L.) MILLSP.), WITH SPECIAL REFERENCE TO ITS PATHOGENICITY

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The interaction of certain soil saprophytes and *Fusarium udum*, the wilt organism of pigeon-pea, with special reference to their effect on pathogenicity, has been studied. The filtrates of *Aspergillus niger*, *Rhizopus nigricans* and mixed filtrates of all the saprophytes inhibited the growth of *Fusarium udum* on solid medium. This inhibition of *F. udum* has been shown to be due to unfavourable reaction of the medium rather than to food exhaustion or the presence of toxic substances. The culture filtrates after passage through soil beds failed to affect adversely the growth of *F. udum* because of the change in pH. Inoculation experiments have indicated that only *Rhizopus nigricans* is effective in reducing the incidence of wilt because of its faster rate of growth. The mixed inocula of the organisms and mixed filtrates of all the saprophytes have also been observed to be effective in reducing wilt incidence. *Aspergillus terreus* appears to enhance the virulence of *Fusarium udum*.

INTRODUCTION

The low incidence of pigeon-pea wilt or suppression of activity of *Fusarium udum* in unsterilized soils was ascribed earlier to the antagonistic effects of the associated soil microflora (Vasudeva & Roy, 1950). It has been reported that neither *Bacillus subtilis* nor its culture filtrate appeared to be effective in checking the parasitic activity of *Fusarium udum* under natural conditions in soil (Vasudeva, Jain & Nema, 1952). Out of the nine fungi isolated along with bacteria (A and B) from unsterilized soils showing low wilt incidence, three fungi, viz. *Alternaria* sp., *Mucor* sp. and *Aspergillus amstelodami*, neither showed faster growth rates nor indicated any inhibition of *Fusarium udum* under laboratory conditions and were therefore not included in further studies. The present investigation was undertaken to obtain more information on the interaction between *F. udum*, the pigeon-pea wilt fungus, and five other soil fungi, viz. *Aspergillus niger* van Tiegh., *A. terreus* Thom., *Cunninghamella elegans* Lendner, *Rhizoctonia bataticola* (Taub.) Butler, *Rhizopus nigricans* Ehrenb., particularly in relation to their effect on the pathogenicity under natural conditions in soil.

Valuable reviews on the subject of biological antagonism have been published by Garrett (1934, 1939), Garrard & Lochhead (1938), Porter & Carter (1938), D'Aeth

(1939), Weindling (1938, 1946, 1950), Waksman (1941, 1948), and Sanford (1946). Endo (1935) has shown that species of *Hypochnus* and *Sclerotium* cease to be pathogenic in the presence of numerous soil fungi and bacteria or their culture filtrates. Sanford & Broadfoot (1931) concluded that the virulence of *Ophiobolus graminis* could be completely controlled by the activities of various soil-inhabiting micro-organisms, and that the filtrates of these organisms were nearly as effective as the living cultures in repressing the pathogen. Henry (1931) studied the natural microflora of the soil in relation to foot-rot of wheat and found that the growth of *Helminthosporium sativum* and *Fusarium graminearum* could be completely suppressed in sterilized soils by the addition of small amounts of unsterilized soil or by the simultaneous inoculation of the soil with a number of fungi and bacteria. Henry's findings were substantiated by Garrett (1934) and Ludwig & Henry (1943). Allen & Haenseler (1935) investigated the antagonistic effects of *Trichoderma lignorum* and reported that it was antagonistic to *Rhizoctonia solani* and *Pythium* spp. responsible for seed decay and damping-off of cucumber seedlings. The parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* has also been reported by Weindling (1932, 1934), Weindling & Fawcett (1936) and Vasudeva & Sikka (1941). Greaney & Machacek (1935) demonstrated by a series of pot experiments that the pathogenicity of *Helminthosporium sativum* on wheat seedlings was suppressed by the antagonistic action of *Cephalothecium roseum*. Tervet (1938) reported that the severity of seedling blight of flax caused by *Fusarium lini* was diminished when the pathogen was accompanied by various other fungi in soil. VanLuijk (1938) suggested that biological control of plant parasites might be obtained by inoculation of soil with specific micro-organisms selected for their antagonistic capacity or by the addition of their growth products.

MATERIAL AND METHODS

Cultures of the fungi *Aspergillus niger*, *A. terreus*, *Cunninghamella elegans*, *Rhizoctonia bataticola*, *R. nigricans* and *Fusarium udum* and of the isolate of *Bacillus subtilis* A used in the previous investigation were obtained from the Indian Type Culture Collection of Fungi. These fungi had been isolated from unsterilized soils showing low wilt incidence, and *Fusarium udum* from the wilted pigeon-pea plants.

Tests for the production of inhibitory substances were made by growing different isolates in potato-dextrose broth for 2 weeks at 30° C. The culture liquids were filtered through Whatman filter-paper (no. 42), sterilized in an autoclave at 15 lb. pressure for 20 min., and assayed as reported earlier by Vasudeva & Roy (1950).

Inoculation experiments were conducted in 12 in. earthen pots, previously washed with 5% lysol solution followed with several changes of tap water and filled with soil autoclaved at 30 lb. pressure for 2 hr. The pot soil was infected with 200 g. (190 : 10) of soil-maize medium on which fungus had been grown in 500 c.c. flasks

at 30° C. for 3 weeks. The surface-sterilized seed of a highly susceptible variety of pigeon-pea, I.P. 69, was used throughout the experimental work.

The pH determinations were made by quinhydrone electrode.

EXPERIMENTAL

Influence of culture filtrates of associated organisms on the growth of Fusarium udum

The soil organisms were tested, individually and in combination, for the production of materials inhibiting the growth of *F. udum* (see Table 1). The fungal filtrates were obtained as already described but *Bacillus subtilis* was grown for only 4 days. Aliquots of 1 c.c. of different filtrates were pipetted out and mixed as required. *Fusarium udum* inoculated on potato-dextrose agar alone, 10 c.c. of potato-dextrose agar alone, and 10 c.c. of potato-dextrose agar diluted with 7 c.c. of water respectively served as controls. Treatment 24 was included since it was thought that mere dilution of the medium with 7 c.c. of different filtrates in treatment 22 might interfere with normal growth of *F. udum*. Treatments 26–30 were included to find out whether the inhibition was due to toxicity or the concentration of the filtrates in different combinations.

Table 1 shows that when the isolates were tested individually only *Aspergillus niger* inhibited the growth of *Fusarium udum*. *Aspergillus terreus* and *Rhizopus nigricans* checked the growth slightly, while *Cunninghamella elegans* and *Rhizoctonia bataticola* had no inhibitory action. Little inhibition was observed in most of the combinations of two isolates except in treatment 8. There was marked inhibition in treatment 21 which represented the combination of all the fungal isolates. The maximum inhibition was obtained with treatment 22, representing a combination of all the fungal isolates and *Bacillus subtilis*. In combinations of filtrates of more than two isolates, e.g. treatments 18–22, the inhibition was more than in treatments 26–30, which is obviously due to difference in concentration of the filtrates. 1 c.c. of the filtrate from treatment 22 appeared to be more effective than the same quantity of the filtrate from other combinations.

As maximum inhibition was obtained with 7 c.c. of the filtrate consisting of all the associated organisms, tests were made with 7 c.c. of the filtrate of each individual organism and with filtrates at different concentrations varying from 10 to 70%. Quantities equivalent to 10, 30, 50 and 70% were pipetted out separately and sterilized in the usual manner. The autoclaved filtrates in different concentrations were then mixed with 10 c.c. of potato-dextrose agar and tested for the growth of *Fusarium udum*. The results are given in Table 2.

Filtrates from *Cunninghamella elegans* and *Rhizoctonia bataticola* did not inhibit the growth of *Fusarium udum*. The filtrate of *F. udum* checked its own growth to a marked degree at all concentrations excepting at 10% level. *Aspergillus niger* exhibited little inhibition at 10% concentration, but marked inhibition at 30%

and complete inhibition at 50%. *Rhizopus nigricans* showed fairly good inhibition at 30%, marked inhibition at 50%, and complete inhibition at 70%. Further, it is clear from the data that there is direct correlation between concentration of the filtrates of *Aspergillus terreus*, *Rhizoctonia bataticola* and *Fusarium udum* and the growth of *F. udum*.

TABLE 1. *Effect of culture filtrates on the growth of Fusarium udum*

No.	Treatment	Diameter of colonies (cm.)	Percentage of growth compared with control
1	<i>Aspergillus niger</i> (A)	3.2	50.8
2	<i>A. terreus</i> (B)	5.0	79.4
3	<i>Cunninghamella elegans</i> (C)	6.0	95.2
4	<i>Rhizoctonia bataticola</i> (D)	6.0	95.2
5	<i>Rhizopus nigricans</i> (E)	5.0	79.4
6	<i>Fusarium udum</i> (F)	3.9	61.9
7	<i>Bacillus subtilis</i> (G)	1.2	19.0
8	A+B	3.1	49.2
9	A+C	4.0	63.5
10	A+D	4.0	63.5
11	A+E	3.8	60.5
12	B+C	4.2	66.6
13	B+D	4.0	63.5
14	B+E	3.9	61.9
15	C+D	4.8	76.2
16	C+E	5.0	79.4
17	D+E	4.1	65.0
18	A+B+C	3.9	61.9
19	A+B+C+D	3.2	50.8
20	A+B+C+D+E	2.3	36.5
21	A+B+C+D+E+F	1.0	15.9
22	A+B+C+D+E+F+G	0.6	9.5
23	Control (P.D.A.)	6.3	100.0
24	P.D.A. + 7 c.c. water	6.3	100.0
25	P.D.A. + 7 c.c. <i>B. subtilis</i>	1.0	15.9
26	1 c.c. from 18	4.5	71.4
27	1 c.c. from 19	4.6	73.0
28	1 c.c. from 20	4.0	63.5
29	1 c.c. from 21	3.9	61.9
30	1 c.c. from 22	3.4	54.0

TABLE 2. *Effect of different concentrations of culture filtrates on the growth of Fusarium udum*

		Concentration of the filtrate and percentage of growth of <i>F. udum</i> compared with control			
No.	Filtrate of	10%	30%	50%	70%
1	<i>Aspergillus niger</i>	47.0	16.7	0	0
2	<i>A. terreus</i>	75.8	59.1	48.5	45.5
3	<i>Cunninghamella elegans</i>	96.9	98.5	96.9	95.5
4	<i>Rhizoctonia bataticola</i>	87.9	80.3	77.3	68.2
5	<i>Rhizopus nigricans</i>	65.2	40.9	10.6	0
6	<i>Fusarium udum</i>	56.1	31.8	25.8	15.1
7	Control	100.0	100.0	100.0	100.0

The inhibition observed in the case of *Aspergillus niger* and *Rhizopus nigricans* was not due to depletion of food nutrients, as is evident from the data set out in Table 3.

TABLE 3. Effect of dilution of filtrates with water and nutrients on the growth of *Fusarium udum*

No.	Filtrate of	Pure filtrate (50 c.c.)		Filtrate-water (25 c.c.-25 c.c.)		Filtrate-medium (25 c.c.-25 c.c.)	
		pH	Growth, <i>F. udum</i>	pH	Growth, <i>F. udum</i>	pH	Growth, <i>F. udum</i>
1	<i>Aspergillus niger</i>	1.72	—	1.93	—	2.12	—
2	<i>A. terreus</i>	5.45	+	—	+	—	+++
3	<i>Cunninghamella elegans</i>	6.39	+	—	+	—	++++
4	<i>Rhizoctonia bataticola</i>	5.89	+	—	+	—	++++
5	<i>Rhizopus nigricans</i>	2.49	—	2.48	—	2.79	—
6	<i>Fusarium udum</i>	5.24	+	—	+	—	++++
7	Control (P.D.S.)	5.74	++++	—	++++	—	++++

— No growth; + Little growth; +++ Fairly good growth; ++++ Good growth.

The pH in the filtrates of *Aspergillus niger* and *Rhizopus nigricans*, which did not support any growth, had fallen to 1.72 and 2.49 respectively. Tests were, therefore, made on adjustment of pH (see Table 4).

TABLE 4. Effect of reaction of the medium on the growth of *Fusarium udum*

No.	Filtrate of	pH	Concentration of the filtrate and percentage of growth of <i>F. udum</i> compared with control			
			10%	30%	50%	70%
1	<i>Aspergillus niger</i>	1.72	46.7	.	.	.
2	<i>A. niger</i>	6.0	95.0	85.0	73.0	70.0
3	<i>Rhizopus nigricans</i>	2.48	63.3	31.7	8.3	.
4	<i>R. nigricans</i>	6.0	91.7	80.0	71.7	65.0
5	Control	6.0	100.0	100.0	100.0	100.0

Table 4 shows that when the pH of the filtrates was adjusted to 6.0, growth was appreciably restored, indicating that the altered reaction of the medium as a result of growth of the two fungi is responsible for inhibition of growth of *Fusarium udum*.

To investigate the reaction of the filtrates in soil, 100 c.c. of filtrates from *Aspergillus niger* and *Rhizopus nigricans* were collected and passed through 1.5 cm. of soil in a Buchner funnel. In addition, 100 c.c. of mixed filtrates of the five organisms and of *Fusarium udum* were also similarly treated. The first few c.c. of the liquid were always rejected. The pH of the filtrates before and after passage through soil was determined and then assayed by the usual method. The results of the tests are given in Table 5.

Table 5 shows that the pH of the filtrates after passage through soil changed from 1.8–3.0 to about 5–5.6. In another test, conducted with a different soil sample, the

TABLE 5. *Effect of passage of fungal filtrates through soil*

Filtrate of	Filtrate before passing through soil		Filtrate after passing through soil	
	pH	% growth of <i>Fusarium udum</i> compared with control	pH	% growth of <i>Fusarium udum</i> compared with control
<i>Aspergillus niger</i>	1.77	50.0	4.97	95.2
<i>Rhizopus nigricans</i>	2.49	69.5	5.20	91.7
Mixed filtrates*	2.96	72.6	5.60	90.3
Control	.	100.0	.	100.0

* Mixture of filtrates of five fungi under investigation and *Fusarium udum*.

pH changed to about 7. The table also indicates that the inhibitory effect of the filtrates is almost lost during the passage through soil. Obviously, therefore, the inhibition effected by *Aspergillus niger* and *Rhizopus nigricans* is associated with the low pH, and when the pH is restored to a favourable range these fungi no longer inhibit the growth of *Fusarium udum*.

To investigate the possibility that growth of *F. udum* in soil might be suppressed by the more rapid growth of the saprophytic fungi, glass tubes (12 × 1 in.), open at both ends, were filled with 100 g. of soil-maize (95 : 5) medium at an initial moisture content of 30%. The tubes were sterilized, inoculated with equal quantities of the fungal cultures, and incubated at 17–21° C. for 3 weeks. Linear growth of the fungi is given below:

Fungus	Average linear growth (mm.)
<i>Rhizopus nigricans</i>	180.0
<i>Cunninghamella elegans</i>	103.5
<i>Rhizoctonia bataticola</i>	58.5
<i>Fusarium udum</i>	58.5
<i>Aspergillus niger</i>	47.0
<i>A. terreus</i>	34.5

In this experiment, *Fusarium udum* was outgrown by *Rhizopus nigricans* and *Cunninghamella elegans* but not by the other species.

Interaction of different soil saprophytes in relation to the pathogenicity of Fusarium udum in soil

The pathogenicity experiments were made to explore the relationship between the severity of the disease caused by *F. udum* and the presence of certain other fungi in the soil. Three series of experiments were set up, as follows:

(a) *Effect of simultaneous inoculation of the pathogen and saprophytes.* The mass inocula of different fungi were raised as described earlier. Details of the treatments are shown in Table 6. Pots containing infected sterilized soil served as controls. After the inoculum had been incorporated, the soil was sown with sterilized

seeds of I.P. 69. The pots were then irrigated and kept in the open; the soil temperature during the experimental period ranged from 16 to 24° C. Observations on the incidence of wilt in different series are recorded in Table 6.

TABLE 6. *Effect of simultaneous mixed inoculations on wilt incidence*

No.	Fungus	Total plants	Plants wilted	Percentage wilt
1	<i>Fusarium udum</i>	23	8	34·8
2	<i>F. udum</i> + <i>Cunninghamella elegans</i>	16	4	25·0
3	<i>C. elegans</i>	7	0	0
4	<i>F. udum</i> + <i>Aspergillus niger</i>	16	4	25·0
5	<i>A. niger</i>	8	0	0
6	<i>F. udum</i> + <i>Rhizopus nigricans</i>	16	1	6·3
7	<i>R. nigricans</i>	8	0	0
8	<i>F. udum</i> + <i>Rhizoctonia bataticola</i>	16	5	31·3
9	<i>R. bataticola</i>	8	0	0
10	<i>F. udum</i> + <i>Aspergillus terreus</i>	15	10	66·6
11	<i>A. terreus</i>	7	0	0
12	<i>F. udum</i> + mixed inocula*	16	2	12·5
13	Mixed inocula	7	0	0
14	Control	23	0	0

* Mixture of *A. niger*, *A. terreus*, *C. elegans*, *R. bataticola* and *R. nigricans*.

The data indicate a tendency towards reduction of disease incidence in mixed inocula, but the results are more marked in treatment 6 (*Fusarium udum* + *Rhizopus nigricans*) and in treatment 12 where mixed inocula of all the fungi have been used. On the other hand, *Aspergillus terreus* appears to enhance the virulence of *Fusarium udum*. All the controls remained healthy.

(b) *Effect of soil saprophytes when added to soil before F. udum.* The inocula of different soil saprophytes and *F. udum* were as usual prepared on soil-maize medium. The soil in the pots was inoculated with the non-pathogenic soil fungi 15 days before being infected with *F. udum*. Pigeon-pea variety I.P. 69 was then sown and the pots were irrigated. The soil temperature during the experiment varied from 16 to 25° C. The results are given in Table 7.

TABLE 7. *Incidence of wilt in mixed inocula with the associated organisms added in advance*

No.	Fungus	Total plants	Plants wilted	Percentage wilt
1	<i>Fusarium udum</i>	19	15	78·9
2	<i>F. udum</i> + <i>Rhizopus nigricans</i>	15	6	40·0
3	<i>F. udum</i> + <i>Cunninghamella elegans</i>	16	12	75·0
4	<i>F. udum</i> + <i>Aspergillus niger</i>	13	10	76·9
5	<i>F. udum</i> + <i>Rhizoctonia bataticola</i>	11	6	54·4
6	<i>F. udum</i> + <i>A. terreus</i>	14	12	85·7
7	<i>F. udum</i> + Mixed inocula	15	8	52·7
8	Control	15	·	·

The results are in general agreement with those recorded in Table 6. The addition of non-pathogenic organisms in advance does not enhance the inhibitory effect.

(c) *The effect of simultaneous addition of the pathogen and the culture filtrates, individually and in different combinations, on the incidence of wilt.* The fungal isolates were grown on potato-dextrose solution at 30° C. for 2 weeks: *Bacillus subtilis* was grown under similar conditions for 4 days. Individual filtrates (200 c.c.) and mixed filtrates of desired combinations were sterilized in the usual manner. After the incorporation of the inoculum of the pathogen from soil-maize medium the pots were sown with seed of I.P. 69 and sterilized filtrates were added. In the control series, however, only filtrates were added. Series containing only sterilized soil also served as control. The temperature during the experimental period was 30–40° C. The results are set out in Table 8.

TABLE 8. *Culture filtrates as influencing wilt incidence*

No.	Treatment	Total plants	Plants wilted	Percentage wilt
1	<i>Fusarium udum</i>	10	4	40.0
2	<i>F. udum</i> + <i>Aspergillus niger</i> (filtrate) (A)	10	3	30.0
3	<i>F. udum</i> + <i>A. terreus</i> (filtrate) (B)	10	4	40.0
4	<i>F. udum</i> + <i>Cunninghamella elegans</i> (filtrate) (C)	9	2	22.2
5	<i>F. udum</i> + <i>Rhizoctonia bataticola</i> (filtrate) (D)	10	3	30.0
6	<i>F. udum</i> + <i>Rhizopus nigricans</i> (filtrate) (E)	9	2	22.2
7	<i>F. udum</i> + <i>F. udum</i> (filtrate) (F)	9	1	11.1
8	<i>F. udum</i> + <i>Bacillus subtilis</i> (filtrate) (G)	9	3	33.3
9	<i>F. udum</i> + A + B + C (filtrate)	10	1	10.0
10	<i>F. udum</i> + A + B + C + D (filtrate)	10	2	20.0
11	<i>F. udum</i> + A + B + C + D + E (filtrate)	9	1	11.1
12	<i>F. udum</i> + A + B + C + D + E + F (filtrate)	10	2	20.0
13	<i>F. udum</i> + A + B + C + D + E + F + G (filtrate)	10	1	10.0
14	Control	10	0	0

This table shows that the filtrates of *Rhizopus nigricans* and *Cunninghamella elegans* reduced wilt incidence. Wilt incidence was appreciably reduced by the filtrates in different combinations, particularly in treatment 9 where *Aspergillus niger*, *A. terreus* and *Cunninghamella elegans* were used. Filtrate of *Fusarium udum* (treatment 7) was as effective as treatment 9. Both the series of controls remained healthy.

DISCUSSION

The cultural filtrates of *Aspergillus niger*, *Rhizopus nigricans* and also the mixed filtrates of all the saprophytes exhibited marked inhibition of *Fusarium udum* on solid medium under laboratory conditions. Sanford & Broadfoot (1931) and Slagg & Fellows (1947) have studied *Ophiobolus graminis* intensively in association with many fungi, bacteria and actinomycetes, and concluded that an individual organism may inhibit the pathogen on one culture medium, stimulate its growth in another,

and may or may not have an antibiotic effect in soil; the same holds for culture filtrates also. Broadfoot (1933) has stated that what is toxic in culture may or may not be toxic in soil. Similar results were also obtained by Cordon & Haenseler (1939), Christensen & Davis (1940) and Katznelson (1942).

The inhibition of *Fusarium udum* by the culture filtrates is not due to lack of food nutrients but to unfavourable pH of the medium. This finding agrees with the observations of several other workers, who have shown that acids are produced by micro-organisms during growth. In a previous investigation (Vasudeva & Roy, 1950) it was stated that *Aspergillus niger* secreted a thermostable inhibitory substance in potato-dextrose broth, but the present study has shown that inhibition of *F. udum* was due only to high acidity of its filtrate.

These high-acid filtrates causing inhibition in culture fail to show any adverse effect on the growth of *F. udum* after passage through a bed of soil because the soil-perfused filtrates are much less acid. In soil, therefore, there appears little probability of any practical control of the disease by the filtrates of these fungi serving as inhibitors.

The pathogenicity tests conducted with simultaneous inoculation of the pathogen and the saprophytes as well as the addition of saprophytes in advance of the pathogen indicated that only *Rhizopus nigricans* is effective in reducing wilt incidence. Its adverse effect on the parasitic activity of *Fusarium udum* may be attributed to its faster rate of growth resulting in suppression of the pathogen. The mixed inocula of all the saprophytes also appear to reduce wilt incidence to a considerable extent. It would, however, be expected that when the associated fungi are pre-inoculated, the activity of *F. udum* would be more adversely affected, but this is not borne out by the experiment and may be due to unfavourable temperature during the winter period. The experiment was started in November and concluded in March: the temperature during most of the time was around 16° C. and rose to 25° C. only during the last two months.

In both series *Aspergillus terreus* appeared to enhance the virulence of the pathogen. Sanford & Broadfoot (1931) found that, when combinations of living cultures of soil fungi and pathogen were added in soil, some isolates were effective in controlling the virulence of *Ophiobolus graminis* while others increased its virulence.

Tests with culture filtrates have indicated that the mixed filtrates in different combinations seem to effect considerable reduction of wilt incidence. The reduction is, perhaps, due to immediate addition of filtrates after inoculation of *Fusarium udum*, since these have direct retarding effect on the pathogen.

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PHYSIOLOGICAL RACES OF *PHYTOPHTHORA INFESTANS*: A COMPARISON OF THE DIFFERENTIAL HOSTS AT THE PLANT BREEDING INSTITUTE, CAMBRIDGE, WITH THOSE OF THE SCOTTISH SOCIETY FOR RESEARCH IN PLANT BREEDING

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At the Plant Breeding Institute, Cambridge, there have been recognized three physiological races of blight (*Phytophthora infestans*), *A*, *B* and *C*; and at the Scottish Society for Research in Plant Breeding, Edinburgh, there have been used five races, *A*, *B*¹, *B*², *C* and *D*, obtained in the British Isles.

It is shown that the two Cambridge types of differential hosts, *AbC* and *ABc* (where *A*=resistant to race *A*, *a*=susceptible to race *A*, etc.), are *Ab*¹*b*²*CD* and *AB*¹*B*²*cD* respectively on the Scottish scheme, and that the Cambridge races *A*, *B* and *C* correspond to the Scottish races *A*, *B*¹ and *C* respectively.

A number of blight isolates were tested on both the Cambridge and Scottish differential hosts. Isolates of race types *A*, *B*¹, *B*², *C* and *D* were found.

The identification and origin of physiological races of blight, and the breeding of blight-resistant potatoes, are discussed.

INTRODUCTION

The existence of physiological races of blight, *Phytophthora infestans* de Bary, was first suspected in England in 1932 (Salaman, 1937; the date of 1936 given in Salaman (1949) would appear to be a mistake) when it was found that certain seedlings, which had been bred by Salaman at Cambridge, and which had remained free from blight for many years, were attacked late in the growing season. This late attack was shown by O'Connor (1933) to be due to a different physiological race of the fungus, which she called race *B*, as opposed to the common type of the fungus, race *A*. In the same year Müller (1932) also found evidence for the existence of physiological races of blight in Germany. In addition to these two races, Black (1952) has found two further races, *C* and *D*, in Scotland and has divided race *B* into races *B*¹ and *B*², the race *B*² having arisen in culture from race *B*¹. Another race, *E*, was obtained by Black (1952) from Tanganyika, and race *E* produced another race, *F*, in culture.

In Germany, Müller has been able to identify some thirty strains of *P. infestans* (Müller, 1950; and see Butler & Jones, 1949, p. 522). It should be noted, however, that Müller uses the reactions of tubers as well as those of leaves to distinguish these races, while in Great Britain leaf reactions only have been used (Black, 1952).

The enumeration of physiological races of any fungus, while it is absolutely necessary to the plant breeder in the task of breeding resistant varieties, is, of course,

a little unreal since it depends upon the standard set of varieties used as differential hosts, the addition of another variety to the standard list often resulting in one or more races being split into two. It is, however, important to have a standard list of varieties so that the physiological races of one investigator can be compared with those studied by others, and it is therefore suggested in this paper that the series of hybrids used by Black at the Scottish Society for Research in Plant Breeding should form the basis for such a list. Fortunately, as is shown later in this paper, the Cambridge Plant Breeding Institute's differential hosts correspond with those used by Black, and Black's series of hybrids have also been used by Mastenbroek (1952) for the comparison of Dutch races of *P. infestans* with the Scottish races.

The comparison of the Cambridge and Scottish material has been made in three ways. First, four races of *P. infestans*, *B*¹, *B*², *C* and *D*, isolated by Black in Scotland, have been used for inoculation of the Cambridge differential hosts; secondly, the three standard races of blight, *A*, *B* and *C*, used in testing the Cambridge breeding material have been inoculated on to four differential hosts used by Black; and thirdly, a collection of blight isolates from England and Northern Ireland have been tested on both the Scottish and Cambridge differential hosts. The two races, *E* and *F*, isolated by Black from Tanganyika have not been used in the present study.

TECHNIQUE

As pointed out in the introduction, only leaves and not tubers have been used in Great Britain for differentiating races of blight. The present work has been done solely with detached leaflets. These detached leaflets are placed in enamel dishes ($10 \times 8\frac{1}{2}$ in. \times $1\frac{3}{4}$ in. deep), sprayed with a suspension of blight spores in water using an atomizer, the dishes covered with a glass plate on which is a sheet of moist blotting-paper, and left in a cold room for 5–7 days. The leaflets are then examined and scored for sporing *v.* non-sporing, no other criterion of susceptibility *v.* resistance being used. Occasionally, when bacterial infections cause the leaflets to rot, the tests have to be repeated.

Although tubers have not been used for differentiating the races of *P. infestans*, they have been used for maintaining the different blight isolates and for producing the spores used in the tests.

THE CAMBRIDGE DIFFERENTIAL HOSTS

Cooper & Howard (1952) showed that the blight-resistant seedlings bred at the Cambridge Plant Breeding Institute, and descended from crosses originally made by Salaman, contain one of two genes, or both genes, for resistance (as judged by the non-sporing character) to *P. infestans*. The first gene gives resistance to races *A* and *C* (Cambridge races) and the second to races *A* and *B*. When both genes are present, the seedlings are resistant to the three Cambridge races *A*, *B* and *C* (e.g. 35/68 and 77/88 of Table 1). The type of resistance found in the Cambridge material and in that of Black and Müller is equivalent more or less to immunity, and it is

due (Müller, 1950) to a hypersensitive reaction, the fungus killing the cells of the resistant types before it can spread to other cells.

In addition to the breeding material two other clones have been used at Cambridge in differentiating races of blight. These are Salaman's 68, which is an example of the first type of resistant seedling bred by Salaman and which is of the same type as the seedlings first attacked in 1932 by the race of blight which O'Connor (1933) called race *B*, and Salaman's 112, which was obtained by him from Müller in Germany and which is an example of Müller's *W* types of blight-resistant potatoes.

The reactions of the Cambridge differential hosts to the Cambridge races of blight and to Black's (Scottish) races *B*¹, *B*², *C* and *D* are shown in Table 1. It can be seen that the Cambridge type *AbC* seedling would be *Ab*¹*b*²*CD* on Black's scheme, and the Cambridge type *ABc* seedling would be *AB*¹*B*²*cD* on Black's scheme. It is also apparent that the Cambridge differential hosts could not differentiate between the Scottish *B*¹ and *B*² races and that they also cannot be used as tests for race *D*.

TABLE 1. *Reactions of the Cambridge differential hosts to the Cambridge blight races and to the Scottish blight races*

Differential host	Type of plant*	Reactions to Cambridge races†			Reactions to Scottish races†			
		<i>A</i>	<i>B</i>	<i>C</i>	<i>B</i> ¹	<i>B</i> ²	<i>C</i>	<i>D</i>
Commercial variety	<i>abc</i>	s	s	s	s	s	s	s
104/14 (or similar type)	<i>AbC</i>	ns	s	ns	s	s	ns	ns
72/105 (or similar type)	<i>ABc</i>	ns	ns	s	ns	ns	s	ns
16/7 (or similar type)	<i>ABC</i>	ns	ns	ns	ns	ns	ns	ns
Salaman's 68	'single' resister	ns	s	ns	s	s	ns	ns
Salaman's 112	<i>W</i> race	ns	s	ns	s	s	ns	ns

* *A* = resistant to Cambridge race *A* of blight, *a* = susceptible, etc. † s = sporling; ns = non-sporling.

Salaman's 68 and Salaman's 112 are both identical with the Cambridge breeding material type *AbC* (or *Ab*¹*b*²*CD* on the Scottish scheme). This is to be expected for Salaman's 68 because the present Cambridge breeding material is related to Salaman's original breeding material before his second line of breeding for resistance to the *B* strain was begun. It is, however, not to be expected for Salaman's 112, which is a Müller *W* race type. The result for Salaman's 112 checks with Black's (1952) result seeing that Black found the variety *Aquila* (see table 1 of Black's paper), which was bred by Müller from his *W* races, to be *Ab*¹*b*²*CD* on his scheme.

THE SCOTTISH DIFFERENTIAL HOSTS

It appears from the results given in Table 1 that the Cambridge *B* strain must be similar to either the Scottish *B*¹ or *B*², and the Cambridge *C* strain to the Scottish *C* strain. This is confirmed by the results given in Table 2 for tests of the Cambridge *B* and *C* strains on the four Scottish differential hosts.

The results given in Table 2 show that the Cambridge race *B* of blight is the same or similar to the Scottish race *B*¹. This was to be expected, since the Scottish race *B*² was not obtained in the field but arose in tests from *B*¹, and since the Cambridge *ABc* type plants from which the Cambridge race *B* was originally obtained

TABLE 2. *Reactions of the Scottish differential hosts with the Cambridge races of blight*

Differential host	Type of plant*	Reaction to Cambridge blight race†		
		<i>A</i>	<i>B</i>	<i>C</i>
Black 1 = 1085 (6)	<i>Ab</i> ¹ <i>b</i> ² <i>CDef</i>	ns	s	ns
Black 2 = 1512c (16)	<i>AB</i> ¹ <i>B</i> ² <i>cDEF</i>	ns	ns	s
Black 3 = 1253a (12)	<i>AB</i> ¹ <i>B</i> ² <i>CDef</i>	ns	ns	ns
Black 4 = 1506b	<i>AB</i> ¹ <i>b</i> ² <i>cdEf</i>	ns	ns	s

* *B*¹ = resistant to race *B*¹, *b*¹ = susceptible to race *B*¹, etc. † s = sporling; ns = non-sporling.

are *Ab*¹*b*²*CD* on the Scottish scheme. The Cambridge blight race *C* is also the same or similar to the Scottish race *C*. This was also perhaps to be expected because, although the Cambridge race *C* used in these experiments was obtained from Cambridge *ABc* type plants growing in Northern Ireland, the original tests to find *ABc* type plants in the Cambridge material were carried out in 1945 with a *C* strain of blight sent by Black from the Scottish Society for Research in Plant Breeding. These *ABc* type plants would, however, have been found in the Cambridge material without the use of the *C* strains from Black since such plants were heavily attacked by blight in Northern Ireland in 1945 and also in many later years (see Table 4).

COLLECTION OF BLIGHT ISOLATES

Isolations of blight have been made for many years from standard commercial varieties and from seedlings obtained at Cambridge in the blight-resistance breeding programme at a number of localities. Some of these results are given in Table 3, and in Table 4 are listed the types of potato from which the blight isolations were made.

The isolations of blight from potatoes grown at Terrington, Cambridge, Moss-side and Stormont require little comment. At all four places isolations of the physiological race types *A* and *B*¹ were obtained from commercial varieties and from *Ab*¹*b*²*CD* type plants respectively, and, with the exception of Cambridge, where no race *C* has so far been observed, isolations of race type *C* have been obtained from *AB*¹*B*²*cd* plants. An isolation corresponding to the Scottish race *D* was obtained only at Stormont on a plant of unknown resistance, but presumably susceptible to this race. Isolations of race *D* would not be expected on the other material because all resistant material bred at Cambridge is resistant to this race.

TABLE 3. *Reactions of isolations of blight on all differential hosts*

Source of isolations*		A. Pilot, <i>ab⁺bc^d</i>	91/37 or 104/14, <i>Ab⁺b⁺CD</i>	72/105 or 78/7, <i>AB⁺B⁺CD</i>	35/68 or 78/88, <i>AB⁺B⁺CD</i>	Salaman's 68, <i>Ab⁺b⁺CD</i>	Salaman's 112, <i>Ab⁺b⁺CD</i>	Black 1, <i>Ab⁺b⁺CD</i>	Black 2, <i>AB⁺B⁺CD</i>	Black 3, <i>AB⁺B⁺CD</i>	Black 4, <i>AB⁺b⁺cd</i>	Race type
From Terrington												
1.	Majestic foliage, 1948	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	A
2.	35/104 foliage, 1948	S	S	ns	ns	S	S	S	ns	ns	ns	B ¹
3.	53/71 foliage, 1948	S	ns	S	ns	ns	ns	ns	S	ns	S	C
From Cambridge												
1.	Tomato fruit, 1950	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	A
2.	Majestic foliage, 1951	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	A
3.	Gladstone foliage, 1951	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	A
4.	100/50 foliage, 1951	S	S	ns	ns	S	S	S	ns	ns	ns	B ¹
5.	Tomato fruit, 1951	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	A
From Moss-side, Co. Antrim												
1.	95/72 foliage, 1950	S	ns	S	ns	ns	ns	ns	S	ns	S	C
2.	Red King foliage, 1951	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	A
3.	Arran Peak foliage, 1951	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	A
4.	Aquila foliage, 1951	S	S	ns	ns	S	S	S	ns	ns	ns	B ¹
5.	100/36 foliage, 1951	S	S	ns	ns	S	S	S	ns	ns	ns	B ¹
6.	100/50 foliage, 1951	S	S	ns	ns	S	S	S	ns	ns	ns	B ¹
7.	95/72 foliage, 1951	S	ns	S	ns	ns	ns	ns	S	ns	S	C
From Stormont, Belfast												
1.	93/10 foliage, 1949	S	ns	S	ns	ns	ns	ns	S	ns	S	C
2.	Arran Victory foliage, 1951	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	A
3.	252/20 foliage, 1951	S	S	ns	ns	—	—	S	ns	ns	ns	B ¹
4.	23/31 foliage, 1951	S	S	ns	ns	—	—	S	ns	ns	ns	B ¹
5.	Seedling ex Müller, 1951	S	ns	ns	ns	ns	ns	ns	ns	ns	S	D
From Dunaney, Co. Antrim												
1a.	35/68 foliage, 1951	S	S	ns	ns	—	—	S	ns	ns	ns	B ¹
1b.	35/68 foliage, 1951	S	S	ns	ns	—	—	S	ns	ns	ns	B ¹
2a.	78/88 foliage, 1951	S	S	ns	ns	S	S	S	ns	ns	S	B ²
2b.	78/88 foliage, 1951	S	S	ns	ns	S	S	S	ns	ns	just s	B ¹
2c.	ex 78/88 on tuber from Calvert	S	S	ns	ns	S	S	S	ns	ns	ns-S	B ²
From Dr K. O. Müller												
1.	Craig's Snow White at Wye, 1950	S	S	ns	ns	S	S	S	ns	ns	ns	B ¹
2.	54/60 at Stickford, 1950	S	S	ns	ns	S	S	S	ns	ns	S	B ²
3.	38/5 at Stickford, 1950	S	ns	S	ns	ns	ns	ns	S	ns	S	C
4.	Aquila tuber at Cambridge, 1950	S	S	ns-S	ns	S	S	S	ns	ns	ns	B ¹

* See Table 4 for further particulars.

The isolations of blight from Dunnaney are of particular interest seeing that they were obtained from two clones, 35/68 and 78/88, which in laboratory tests are resistant to races *A*, *B*¹, *B*², *C* and *D*. Plants of these clones were attacked by blight in the late September of 1951. The blight was transferred from their foliage to

TABLE 4. *Resistance types of plants from which blight isolations of Table 3 were made*

Isolation (see Table 3) Resistance of plants or plants from which bred*

Terrington

- 1 Majestic, *ab*¹*b*²*cd*
- 2 35/104, Cambridge breeding material, *Ab*¹*b*²*CD*
- 3 53/71, Cambridge breeding material, *AB*¹*B*²*cD*

Cambridge

- 1, 5 Tomato fruit, not tested
- 2, 3 Majestic and Gladstone, *ab*¹*b*²*cd*
- 4 100/50, Cambridge breeding material, *Ab*¹*b*²*CD*

Moss-side

- 1, 7 95/72, Cambridge breeding material, *AB*¹*B*²*cD*
- 2, 3 Red King and Arran Peak, both *ab*¹*b*²*cd*
- 4 Aquila, German var. bred by K. O. Müller, *Ab*¹*b*²*CD*
- 5, 6 100/36 and 100/50, both Cambridge bred material, *Ab*¹*b*²*CD*

Stormont

- 1 93/10, Cambridge breeding material, *AB*¹*B*²*cD*
- 2 252/20, seedling ex Müller *W* race, *Ab*¹*b*²*CD*
- 3 23/31, seedling ex Müller *W* race, *Ab*¹*b*²*CD*
- 4 Seedling ex Müller, *Ab*¹*b*²*CD*?

Dunanney

- 1*a*, *b* 35/68, Cambridge breeding material, *AB*¹*B*²*CD*
- 2*a*, *b*, *c* 78/88, Cambridge breeding material, *AB*¹*B*²*CD*

Müller's

- 1 Craig's Snow White, *Ab*¹*b*²*CD*
- 2 54/60, Cambridge breeding material, *Ab*¹*b*²*CD*
- 3 38/5, Cambridge breeding material, *AB*¹*B*²*cD*
- 4 Aquila, German var. bred by K. O. Müller, *Ab*¹*b*²*CD*

* *A*=resistant to race *A*, *a*=susceptible to race *A*, etc., as in Tables 1-3.

tubers by Mr J. Bankhead, who then sent some of the infected tubers to Cambridge. These isolates were maintained in tubers during the winter and used for tests in the spring of 1952. It was expected that they would be found to be of a new physiological race. However, as the results in Table 3 show, they appear to be either *B*¹ or *B*² type races. Also, they did not infect detached leaves of 35/68 and 78/88 in numerous laboratory tests. It was possible that they might have become contaminated at Cambridge in the winter with a *B*¹ strain, but this seems unlikely, especially as an isolation kept over the winter by Mr E. L. Calvert at Queen's University, Belfast, behaved in a similar way when used for tests at Cambridge. Difficulty was also found at Cambridge in deciding whether the isolates from

78/88 were of race type B^1 or B^2 , since in some tests they did not spore on Black 4, in other tests they produced a few conidiophores, and in still other tests they produced a large number of spores. Attempts to isolate a culture which would always spore on Black 4 were unsuccessful.

Dr Müller's isolate from 54/60 (an Ab^1b^2CD type) appeared to be of race B^2 and not race B^1 . This is in marked contrast to all other isolates from Ab^1b^2CD plants, which were always of race type B^1 . Secondly, the isolate from a tuber of Aquila (an Ab^1b^2CD type—see Black, 1952, table 1) produced sporing in some tests on 72/105 but never on 78/7. There was no reason to suspect that it was contaminated with a race C isolation. On the other hand, after subculturing for some five or six tests on Ab^1b^2CD type plants it lost this ability to spore on 72/105 (AB^1B^2cD).

Taken as a whole the results for the collection of blight isolations agree with the previous results given in Table 1 and 2 in showing that Cambridge AbC type and ABc type plants correspond to the Scottish types Ab^1b^2CD and AB^1B^2cD respectively. They do, however, also suggest that it is not always easy to identify an isolation as B^1 or B^2 , which is perhaps not surprising since Black obtained B^2 from B^1 in culture, and that other subtypes of race B may occur (e.g. the isolate from Aquila tuber).

DISCUSSION

The number of physiological races in Great Britain

The number of races which can be found depends both upon the number of differential hosts used and upon the number of different types of resistant plants from which the blight collections are made. The importance of the number of hosts has been shown by comparing the Cambridge differential hosts, which will identify three races, with the Scottish hosts, which will identify five. The importance of the types of resistant plants has been shown by finding that only races of the A , B and C type are obtained from commercial and Cambridge resistant material, the single isolation of race type D having been obtained from other material.

The only difficulty in placing blight collections in the various physiological races has been in deciding whether a collection was of race B^1 or race B^2 , which arose from race B^1 in culture. Another difficulty was that races B^1 and B^2 were obtained late in the season from clones which are usually resistant to races A , B^1 , B^2 , C and D . It might be expected that blight on such plants would be of another race, but all tests suggest it to be either B^1 or B^2 . The fact that such plants can be attacked late in the season is of considerable importance in breeding for blight immunity and in considering the origin of new races of blight.

Field observations in Northern Ireland on clones which in laboratory tests would all be classified as Ab^1b^2CD have suggested that there may exist a whole series of races of the B type, since, under natural infection, such clones show very different degrees of attack. It has not, however, been possible in laboratory tests to show

that there are more than the B^1 and B^2 races found originally by Black. It may be therefore that the different clones differ not in their major genes for resistance, but in modifying genes for resistance as suggested by Black (1952, p. 345).

Comparison of differential hosts

The Cambridge AbC types, the Scottish Ab^1b^2CD type, the Salaman original resistant clones ('single' resisters) and the Müller W race potatoes all agree in their reactions to the A , B^1 , B^2 , C and D races of blight. This could be explained readily if it was known that these four types of resistant potatoes had a common origin from the same stock of *Solanum demissum* or from different stocks of *S. demissum* carrying the same factor for resistance. It is not, however, possible now to check this explanation, particularly since the origin of the Müller W race potatoes is obscure.

Similarly, the agreement between the Cambridge ABc type differential hosts and the Scottish Ab^1b^2CD type may also be due to their both having received the same factor for resistance from similar *S. demissum* stocks.

Origin of the different physiological races

It has been observed both in England and Northern Ireland that Cambridge AbC and ABc type plants grown for only one year in certain localities (e.g. Claudy, Co. Londonderry, 1947; Terrington, Norfolk, 1948; and Moss-side, Co. Antrim, 1950) have been attacked by the B and C physiological races of blight respectively. Tests made of blight isolates also show that even in adjacent drills a normal commercial variety, an AbC type clone and an ABc type clone would appear to be attacked by race A , race B and race C respectively.

The two results given in the previous paragraph could be explained in three ways. First, it may be that the so-called race A found on commercial varieties is a mixture of, for example, 99% race A , 0.5% race B and 0.5% race C . The AbC and ABc plants would then pick out the race B and C components from the mixture. It should be possible to test this explanation by spraying blight isolations from commercial varieties on to a large number of AbC and ABc type leaflets. Results of a few relatively small experiments at Cambridge have so far been negative.

Secondly, it is possible that spores of race B and race C arise by mutation from race A . This would not require a very high mutation rate since the number of spores produced is very large. Also, it has been suggested by Catcheside (1951) that it is a general rule that such new types of a fungus are recessive to the common type (the other general rule suggested by Catcheside, that resistance of the host plant to a particular race of the fungus is a dominant character, does hold for blight-resistance in potatoes).

Thirdly, it may be that the AbC and ABc type plants in some way 'train' the blight fungus so that it changes from what is identified as an A race into what are identified as B and C races respectively. Both Reddick & Mills (1938) and de Bruyn

(1947) have claimed to have brought about such a training of the fungus under laboratory conditions.

There would appear to be no reason for suspecting that new blight races will normally be produced by sexual reproduction, since, although oospore formation has been observed, it does not appear to be a normal part of the life cycle of blight. It would, therefore, appear that the mechanism of origin of new races of blight is not understood.

The possibility of breeding blight-resistant potatoes

All the blight-resistant potato varieties that have been introduced in the past 10–15 years are immune only to certain races of blight (Müller & Black, 1951). Thus Craig's Snow White and Craig's Bounty, Aquila and other German varieties, and Kennebec and other American varieties are all susceptible to race *B*¹. Since race *B*¹ seems to occur in many places as soon as *AbC* type clones are grown, it is almost certain that these new varieties will not prove field-resistant. However, there has recently been introduced (Anon. 1952) the variety, Pentland Ace, which is field immune to races *A*, *B* and *C*, but this has not yet been grown on a wide acreage.

The type of blight resistance so far discussed is the hypersensitive type derived generally from the wild species *S. demissum* (Müller, 1950). There are, however, other types of blight resistance known, even in the commonly cultivated varieties. There may be resistance to infection, or there may be resistance resulting from very slow growth and poor fructification of the fungus in certain varieties (cf. Müller & Munro, 1951). It would appear that new resistant varieties would be more valuable if they had these other types of resistance as well as the hypersensitive type of immunity, since, if they have, it should take a much longer time for any new race of blight produced to become abundant. Their value, however, could only be finally judged by trials in areas where blight attacks are severe.

My thanks are due to Dr W. Black of the Scottish Society for Research in Plant Breeding, Edinburgh, for sending me the four Scottish differential hosts and the four Scottish blight races; to Mr J. Bankhead of the Northern Ireland Ministry of Agriculture for blight isolates; and to Dr K. O. Müller of the National Institute of Agricultural Botany, Cambridge, for blight isolates.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

General Meeting of the Association held at Imperial College, London, on Friday,
27 February 1953; the President, Mr R. W. Marsh, in the Chair.

Symposium on the assessment of plant pests and diseases

The following papers were read and discussed:

1. Some recent developments in fungus disease survey work in England and Wales. By Mr E. C. LARGE.
2. The measurement of losses caused by apple-tree diseases, with special reference to apple scab. By Dr H. E. CROXALL, Mr D. C. GWYNNE and Mr J. E. E. JENKINS.
3. Assessment of disease incidence in the sugar-beet crop. By Dr R. HULL.
4. Field technique in pest assessment. By Mr A. H. STRICKLAND.
5. The assessment of injury by seedling pests of sugar beet. By Mr F. G. W. JONES.
6. The assessment of damage caused to some brassica crops by the cabbage-root fly. By Mr D. W. WRIGHT.

SOME RECENT DEVELOPMENTS IN FUNGUS DISEASE SURVEY WORK IN ENGLAND AND WALES

BY E. C. LARGE

Plant Pathology Laboratory, Harpenden, Herts

(With 1 Text-figure)

My contribution to-day will be in the nature of a progress report. When Mr W. C. Moore gave his Presidential Address to this Association in 1949 he reviewed the wealth of information on plant diseases that had been extracted from the monthly reports sent into the Plant Pathology Laboratory, without break, for the past thirty-six years. But his first words were a challenging confession of a gap in our knowledge. A great number of reports of individual occurrences of plant diseases have told us what diseases we have in this country, and a little about their distribution. But they do not, and they cannot, tell us how *many* crops are affected or how severely. To obtain this kind of quantitative information special surveys are needed; and here and there they have been made—witness Dr Hull's fine work on the incidence of virus yellows in sugar beet, about which we shall hear later this morning—but in the main, and in common with most other countries in the world, we are still lacking the information upon which reliable estimates of disease losses can be based; all is still guesswork.

Following closely upon Moore's exposure of our ignorance, new appointments were made at the Plant Pathology Laboratory. I joined the staff in July 1950 to promote quantitative work on the plant disease side, and eventually to assist Mr Moore and Dr Thomas as editor of a new plant pathological journal (*Plant Pathology*) which was to have, as one of its main

objects, the prompt publication of the results of quantitative survey work on both plant diseases and pests. A little later, in 1950, Mr Strickland joined us to promote the work on the entomological side, and I think that on the whole he has the harder job, for he has to deal with multitudinous insect populations as well as the damage they cause. But he will be telling us about his work this afternoon, I will confine myself to mine.

Now, although I have worked in plant pathology for over twenty years—in commerce, on my own, and in the advisory service—I began life as an engineer; and my outlook will always

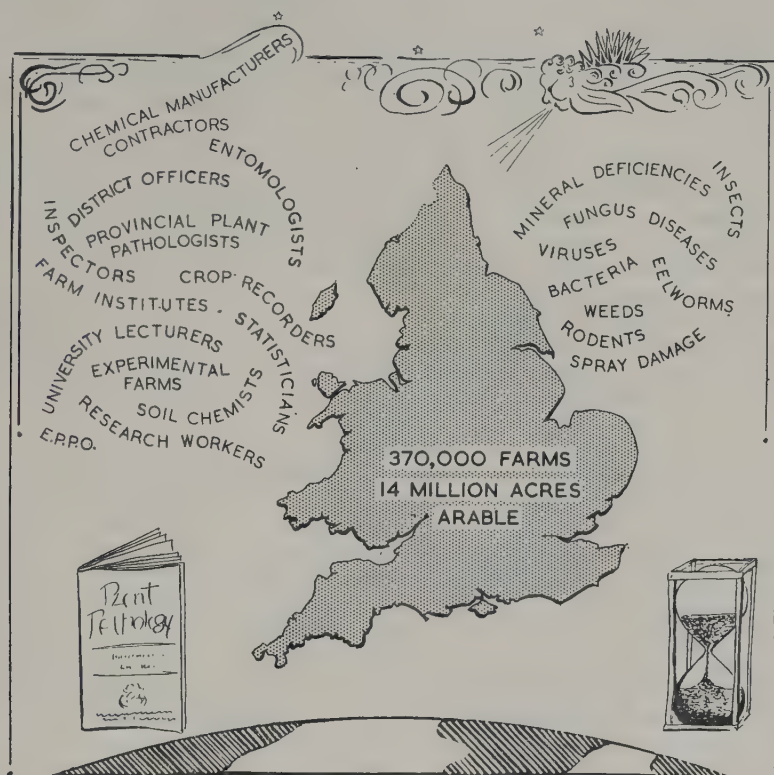


Fig. 1.

be that of the engineer, of the man whose job it is to making something that will *work* out of the materials he finds available. Let me now show you *my* impression of the job that lies before us to be done, and of those who are concerned in doing it, or in reaping the results of it (Fig. 1). In England and Wales we have over 370,000 farms, large and small, and some 14 million acres of arable land under crops or rotation grasses. The job, in the end, is to obtain a true estimate of the significance to the farmer, and to the national economy, of each of the principal plant diseases affecting the crops over the whole of that very large area. It is a mighty undertaking, and at best we can only hope to make a good beginning, in what is left of *our* working lives. On the one side we have an ecological jungle of plant pathogens and pests, rarely occurring singly or acting alone, from amongst whose effects we must distinguish those caused by the agent under survey. Without accurate diagnosis our surveys may be worse than useless. Then, on the other hand, we have what appears to be a confusion, but is in fact a very delicately balanced free association, of many groups of workers from

amongst whom we must find our surveyors. We must know the life history of our pathogens and their effects on the gross morphology and development of our crops plants. We have to know much more than the theory: our keys must be based on critical details and appearances that can only be picked out after close and devoted study of the growing plants in the field, and we have to think of our plants, not as they are at one stage only, but as complete entities in time from planting to harvest. Time is a very real dimension in this work. Then we must know the life purposes of our potential surveyors, and shape our requests for their aid in such a way that participation in the surveys helps forward the work in which they are otherwise engaged. We must proceed in a curiously English way, asking for much less than we know we shall receive, and often doing one thing under cover of another. While, all the time, two presiding daemons confuse and complicate our undertaking: our infernal weather, which sees to it that no two seasons are ever quite the same, and the great diversity of our soil types.

We have no workers solely engaged on plant disease surveying; and it seems immediately obvious to me that if we want to survey any substantial part of those fourteen million acres for plant diseases, we must graft our surveying, or our 'disease assessment', on to other work—on to any and every kind of work, advisory, investigational or educational, that already demands frequent inspection of growing crops. So I am a hitch-hiker, ready to travel on any band-waggon that is going my way.

Let me now give you a few examples of survey work that we have recently put into operation by pursuance of this amiable policy; and let me say at once that by 'we' I mean the Conference of Advisory Plant Pathologists in the National Agricultural Advisory Service, with whom we at the Plant Pathology Laboratory are united in faithful and fruitful wedlock.

When I went over from one side of this partnership to the other, in 1950, the Conference, in collaboration with the Agricultural Branch of the Meteorological Office, had just started an investigation on Potato Blight Forecasting. I was asked to look after this work, on our side, and nothing could have suited me better. First, it brought me into day-to-day consultation with Mr L. P. Smith and helped me to realize something of the extent of the resources possessed by the Agricultural Branch of the Meteorological Office, for obtaining just the kind of weather data so indispensably necessary in plant disease survey work. I found an existing, growing and enthusiastic organization, dealing with this highly specialized branch of applied physics, and ready and willing to help us in every possible way. Whatever our other difficulties might be, the *weather* was going to be well and truly observed. A whole country-wide network of synoptic weather stations was already participating in the blight forecasting investigation.

Then the object of this investigation was to relate the occurrence of Beaumont periods at all these stations with the dates of outbreak of blight in their areas. The plant pathologists, aided by district officers and research workers, sent in many hundreds of observations on date of outbreak from all parts of the country; and in 1950, which was a 'blight year', we had, for the first time in our agricultural history, a map fairly showing the time at which blight started in all parts of England and Wales. Incidentally, the success of the forecasting was extremely encouraging, but as the full results of the first three years' work on that will be published in the March issue of *Plant Pathology* (Large, 1953), I will not go into it now. I am showing the map because it gives the zoning of the country for date of outbreak—the first step in the systematic surveying of potato blight.

The next step was to follow up the observations on 'outbreak', and to obtain some information on the *progress* of the attack in the different regions. Here we had the advantage of having done some real spadework in the past. Back in 1941 a Disease Measurement Committee of the British Mycological Society worked out a simple key for the rapid visual assessment of potato blight on the haulm. Beaumont and I used this key in our work on potato blight in Devon and Cornwall during the years 1941–6, and it had been well tested in use, both there and elsewhere. The next thing we did was therefore to issue this key to all

plant pathologists and district officers in the N.A.A.S., as well as to N.I.A.B. crop recorders, and Farm Institute and Experimental Farm workers, and to ask them to make fortnightly assessments of blight, by the key, wherever they had opportunity. The plant pathologists helped all the observers in the recognition of blight—which is sometimes all too closely resembled by the effects of potash deficiency—and critical observations were checked and confirmed microscopically. All the collaborators found the field work very interesting; and it has helped to train those who were not mycologists in the recognition and understanding of this important plant disease.

So, in 1950, and in the succeeding years of the forecasting survey, we were able to plot progress curves, showing the course of haulm destruction by blight in the several regions. These curves bring out the great differences in the time at which growth is stopped by blight, as between one part of the country and another, and between one year and another.

Thanks to an original idea of Mr Beaumont's, based on the classical work of Paul Murphy, we have a means of deriving from these progress curves an estimate of the mean loss of yield caused by blight in each year and region (Large, 1952). When the destruction of the foliage by blight reaches about 75 % by our key, tuber production stops. If, therefore, we project upwards from the 75 % point on our progress curve to another curve—derived from bulking trials—showing the loss of potential crop when growth is stopped at various times in the growing period, we can read off an estimate of the mean probable loss of crop that was caused by the blight.

That is the method in outline. Of course, there are corrections to be made; the curves must be calibrated and the method checked extensively against the results of spraying trials at a number of key centres, and over a number of years. But this work is well under way and, in the not too distant future, we shall have a means of obtaining what we have so long needed: a reliable zoning of the country into regions in which routine protective spraying is, and is not, economically justified, and of giving farmers in each region an estimate of their mean expectation of gain or loss from spraying, over a period of years.

At this point what we have been calling 'disease measurement' or 'disease assessment' enters a new phase. It becomes a systematized and specialized method of mensuration with a derived superstructure. In short, it becomes a branch of plant pathological science, and I think it deserves a better name. The name that I would now, and hereby, propose for it is 'Plant Pathometry', from *pathos*, disease or suffering, and *metron*, measure.

Before leaving our blight forecasting investigation, which as you will see we have transformed into a survey with a scope far beyond anything that was originally intended, I should like to refer to the map constructed for 1952, with its progress curves. This map shows that in the particular year 1952, protective spraying might have paid in the western half of the country, and in parts of the south, but that in the east it was a waste of labour and materials. In some other years, as in 1950, protective spraying was needed in the east. How often is it needed? That we shall see; the results of our investigations will be published when they are ready, and without delay, in *Plant Pathology*. For them we must wait.

Now let me turn to another investigation, on to which we have tacked a little of our practical 'plant pathometry'. In 1951 there was a world shortage of sulphur, and with the plant pathologists and crop husbandry officers of the N.A.A.S. we conducted trials, at twelve centres in England and Wales, of substitutes for sulphuric acid in the destruction of potato haulm (Large, 1952). The trials are continuing for three years. Blight progress curves are being obtained for all the crops in these trials, and the curves for the untreated controls are clearly revealing the blight conditions under which haulm destruction is, and is not, of value for the prevention of blight in the tubers. Where the crop would otherwise be lifted while the haulm is still green and with active blight on it, the operation is worth while, even almost essential. But where, as it more often happens, the haulm would have been dead anyway before lifting, the operation has had little or no effect on the amount of blight in the tubers. Usually it is done so late that there is plenty of opportunity for infection of the tubers in the soil *before* the date of haulm destruction, especially where the soil cover is none too

good; and the ideal way of ensuring that there is no blight in the tubers is to keep it off the haulm up to the date of haulm destruction or natural dying down. We have known all this in the past, but the application of pathometric methods exposes what happens with great clarity, and provides graphical records of experience in the past for the guidance of advisers in the future.

Now for another piece of work of special interest in that it permits us to compare results obtained by two different methods of surveying. For some years before this work started, in 1951, the grassland officers of the N.A.A.S. had been coming across a number of cocksfoot seed crops heavily infected with choke (*Epichloe typhina*) and this gave them the impression that the disease was very widespread and destructive. They pressed the plant pathologists to investigate the matter, and in 1951 we began two surveys (Large, 1952). One, an *extensive* survey covering *all* the crops entered for certification, in which the inspectors simply assessed choke in each crop as 'absent', 'occasional', 'frequent' or 'severe'; and an *intensive* survey, in which the plant pathologists examined a random sample of about one-tenth of the crops, and made accurate sample counts in each of them. Here, as in every plant disease survey, there were the symptoms of the disease-to-be-observed, and the masquerading appearances that had to be distinguished from it. There were tillers blind by reason of choke, and tillers blind from other causes. So it seemed at first that the plant pathologists would have to count not only the tillers showing the external stroma of the fungus, but also those with no external symptoms, but with *Epichloe* mycelium in the pith—an extremely tedious business. The first important finding of the survey was that the task had been wrongly conceived. Fortunately, it was found that if the inspection was left until all the healthy flower heads had emerged, there were in fact only three kinds of tillers on cocksfoot plants: healthy flowering tillers, choked tillers with external stroma, and vegetative tillers which would not be ready to throw up a flowering head until the following season. Hence all that was necessary was to count the number of healthy and of obviously choked tillers and the work was greatly simplified.

In the two years 1951 and 1952 the inspectors assessed choke in 1420 crops and the plant pathologists in 158. By their very rough method, and for all the personal differences in interpretation of what was meant by 'occasional', 'frequent', and 'severe', the inspectors provided an excellent picture of the true state of affairs. There were no regional or varietal differences; the incidence of choke depended upon the age of the stand. There was practically no choke in the first-year crops, but it appeared in those of the second year, became progressively worse in the third and fourth years, but only in the fifth and sixth years was any considerable proportion of the crops severely affected. The intensive survey revealed the same trend, but in quantitative terms. In the two years, 1951 and 1952, there were only forty-one crops altogether that had been left down for as long as five or six years, so that the severe choke which had caused so much concern, was in fact only occurring in about 1% of our cocksfoot seed crops, and even so only in old crops, many of which were weedy and starved and had become unproductive for reasons other than choke.

Next a piece of survey work, that has been running only one year, on a disease about which all too little is known: common scab of potatoes. We have a Disease Assessment Committee of the N.A.A.S., successor to that started by the British Mycological Society, whose function it is to draw up keys for the rapid assessment of plant diseases, and to advise the Conference on the methods to be adopted in its surveys. Various pieces of homework are undertaken by members of this Committee, and one that fell to my lot was to devise a provisional key for common scab. I examined dozens of samples of scabbed tubers, and entertained myself in trying out at least seven different ways of estimating, first the surface area of a potato tuber, and then the proportion of that area covered by scab lesions. One way was to stick black paper disks of known area on to potato tubers, so that they covered known proportions of the surface and then to contemplate the result. It is a matter of training the eye, and such training is necessary for all visual assessment of degrees of spotting or blemish. Dr Croxall will be going further into this matter of the assessment of spot and blemish, in his paper on

apple scab. All I need say is that eventually I came to the conclusion that a very simple key diagram was all that was required for common scab of potatoes, and a method based on this was put out for trial. In fact a very substantial survey was made with the aid of the diagram last year by the Potato Division of the Ministry of Food, who gave us most stalwart help, by making supplementary weighings of tubers showing two degrees of scab, in their crop check weighings all over the county. Thus we have figures for the incidence of scab in some 2400 potato crops, very carefully selected to be representative of both large and small fields and of the several types of soil, in each county. The results show obvious regional differences in the incidences of scab, and also marked varietal differences. The collation of the mass of results for 1952 is not yet complete, there will be much work to do on interpretation, and we shall almost certainly need similar surveys in other years.

Finally, let me say a little about the survey which we have most recently started, and our most difficult one so far, which is an attempt to ascertain the real extent of the damage caused by clover rot throughout England and Wales, and to distinguish that damage from the effects of clover eelworm and other disorders. Here we have not had the advantage of being able to attach our survey work to another investigation; we have had to launch the survey on its own. The history of the survey so far, is, I think, typical of what will happen whenever that hard way has to be taken. First there have been the enthusiasts, the true originators of the survey, who at their own initiative have done a real piece of pioneering work in their own areas. In this case the pioneers were the plant pathologists in the East Midland and Eastern Provinces. They tackled the problem in different but complementary ways, and devised keys or methods of assessment to meet their own needs. The methods and the results obtained by them were then examined by the Disease Assessment Committee, and proposals were drawn up for a trial run in other areas. The trial run was made; the methods were revised; and only after that did the Conference decide to undertake a full-scale co-operative survey over the whole country. The apportionment of the work between the several groups of observers: the plant pathologists, the district officers, and the entomologists was debated at length. A compromise was made between the kind of sampling that would please the statisticians, and what the district officers could reasonably be expected to do without dislocating their other work. The record forms to be used were drafted over and over again, weeks of work went into that part of the job alone, but at length everybody was more or less satisfied, and three thousand forms were issued and distributed to the observers, with careful explanatory memoranda. Now the field work is under way, and the one thing certain is that as it proceeds year by year it will change its course. Each year the completed record forms will come back to our Laboratory for collation. Interim reports on them will be drawn up for the Conference, and our very shrewd and experienced provincial plant pathologists will check and debate the collations. Plans will be made for the next year's work, everybody who can contribute anything useful will be consulted, and subject to the inescapable limitations of space and time, all the results will be put promptly before you, in the pages of our journal *Plant Pathology*. That is how we are conducting our survey work.

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THE MEASUREMENT OF LOSSES CAUSED BY APPLE-TREE DISEASES, WITH SPECIAL REFERENCE TO APPLE SCAB

BY H. E. CROXALL, D. C. GWYNNE and J. E. E. JENKINS

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The work we have started on assessment of damage done by diseases of apples has two main objectives; the first is to provide methods by which the amount of disease present may be estimated rapidly, but at the same time with reasonable accuracy in the field; the second is to correlate the amount of disease present with loss in quantity and quality of the crops. So far we have not considered lethal diseases such as *Armillaria* root rot or silver leaf, worthy though they are of investigation, but have been concerned mainly with apple scab and brown rot.

In the case of apple scab there are two phases of the disease, on the leaves and on the fruit. Kearns, Marsh & Martin (1945) have referred to Tehon and Stout's method of estimating the amount of disease present on the leaves by comparing sample leaves with standard diagrams, showing a known percentage of leaf surface covered by lesions. In our work we have found that these diagrams are used in different ways by different operators. Some match their sample leaves to the nearest diagram, for example, 0, 1, 5, 10, 25 or 50 % leaf area covered by scab. The number allotted to each percentage are multiplied by that percentage, the totals added and divided by the grand total of leaves examined. For example

$$\text{Mean percentage leaf area scabbed} = \frac{a0 + b1 + c5 + d10 + e25 + f50}{a + b + c + d + e + f}.$$

Unfortunately the categories used are not in arithmetical progression so that the leaves estimated as 1 % will cover the range from 0 to some point between 1 and 5 %. Where this point lies will depend upon the observer; it may be at the arithmetical or the geometrical mean between 1 and 5, and it may even vary on different occasions. This applies to all the categories used and may become more important as the interval between them increases. To avoid this difficulty we have adopted the method of using the standard diagrams as the limits of categories. For example, one category would be between the limits of 1 and 5 %, and any leaf with scab of just over 1 % to just under 5 % would fall into this category, and the number of leaves so infected would be multiplied by the mean which is 3. With this method the categories are 0, 0-1, 1-5, 5-10, 10-25, 25-50 and 50-100, and the formula for the mean percentage leaf area scabbed is

$$\frac{a0 + b0.5 + c3 + d7.5 + e17.5 + f37.5 + g75}{a + b + c + d + e + f + g}.$$

In the main we not only have a clear line of demarcation between the categories, but we also introduce an extra category in the important lower range of infection.

This method is admirable for research purposes, but is too time-consuming for a rapid field survey. We have, therefore, attempted to devise a word picture key similar to the one used successfully in potato blight survey work.

Key for apple scab on leaves

On each tree, five runs of branches, chosen at random round the tree, are taken as sampling units. A 'run' of branches consists of a main branch and all the laterals, sublaterals and spurs on it. The appearance of all five units is taken into account in assessing the percentage of scab on the tree as a whole. Where possible, recordings should be made on ten trees of each variety.

Scab (%)

- 0.01 Occasional units show one or two infected leaves
- 0.1 Scab spots on one or two leaves in each unit
- 0.25 Every unit shows up to five small spots or their equivalent on about one-quarter of the leaves
- 0.5 Every unit shows five small spots or their equivalent on about half the leaves
- 1.0 Majority of leaves infected, a few with about 25 % of their area covered with spots
- 5.0 Almost every leaf infected with the scab areas covering approximately 25 % of the leaf surface on about one-quarter of the leaves
- 10.0 Every leaf infected with the scab areas covering approximately 25 % of the leaf surface on half the leaves
- 25.0 Every leaf infected with the scab areas covering 50 % of the leaf surface on half the leaves
- 50.0 All leaves infected, with the scab areas covering almost the entire leaf surface on about half the leaves

Notes

- (1) If only a single observation can be made, it should be at three to four weeks after petal fall.
 - (2) The incidence of scab on fruitlets should be noted and recorded as nil, slight, moderate or severe.
 - (3) Note should be taken of the general condition of the trees and their management, including ages, rootstock, other diseases and pests, whether grassed or cultivated or intercropped, distance apart of trees, and spraying programme, if any.
- (This key is slightly modified from that printed in *Plant Pathology* Croxall, Gwynne & Jenkins (1952a) in that the 0.05 % description is now given as 0.1 %.)

As shown by Croxall *et al.* (1952a) this method has given results of the same order as those obtained by the leaf-sampling technique but it requires further testing under a wider range of conditions.

As fruit is not always present estimation of amount of scab on the leaves may be essential in evaluating the relative efficiency of fungicides or comparing the intensity of the disease in different seasons, but it does not appear possible to equate the results with the economic loss. The amount of scab present on the leaves may bear no relationship to that on the fruit due to differences in susceptibility of leaves and fruit even on the same tree, and the fact that conditions during fruit formation may not be so favourable to infection as they may have been for leaf infection earlier in the season. Moreover, the damage to the leaves is unlikely to affect the weight of fruit appreciably as it seldom exceeds 2-3 % of the leaf-surface area. Therefore, if possible, assessment of scab should be made on the fruit.

As described by Croxall, Gwynne & Jenkins (1952b) this again may be done by the comparison of apple samples with standard diagrams. However, this again is too slow for rapid field survey and also involves picking the fruit so we have devised another word picture key.

Key for apple scab on fruit

If leaf assessments have been made, the same trees should be used. If not, recordings should be made, where possible, on ten trees for each variety, uniformly distributed throughout the plantation. On each tree five runs of branches, chosen at random round the tree, should be taken as sampling units. Assess each tree separately and take the mean.

Scab (%)

- 0.01 Occasional units show one or two fruits infected
- 0.1 Small scab spots on one or two fruits in each unit
- 0.5 Small scab spots on about one-quarter of the fruits in each unit
- 1.0 Up to five small scab spots on about one-half of the fruit in each unit. No cracking
- 5.0 Scab spots on about three-quarters of the fruit in each unit. Occasional fruits may be cracked
- 10.0 Every fruit in each unit shows scabbed areas with a few cracking

- 25° Every fruit in each unit shows large scab spots with up to a quarter of the fruits cracked. Fruit more variable in size and, on the whole, slightly smaller than fruit from healthy trees
- 50° Every fruit in each unit shows large scab spots with about one-third of the fruit severely cracked and of non-marketable grade. Fruit generally smaller than that from healthy trees
- 75° Every fruit in each unit shows large scab spots with about one-half of the fruit severely cracked and of non-marketable grade. Fruit appreciably smaller than that from healthy trees

Blemishes other than scab, such as capsid damage, brown rot, etc., should be noted.

We are now investigating whether the mean percentage scabbed area of the fruit can be related to the grading of the crop according to the recommended grades published in Marketing Guide no. 30 (1951). The results obtained (Croxall *et al.* 1952*b*) are promising but many more samples must be examined.

With samples of picked fruit a standard diagram method using the diagram as the limits of categories appears to offer the best possibility of obtaining information on the loss of crop. The number of diagrams used will depend upon the time available, but should include those representing the maximum permissible blemish for each of the recommended grades of fruit. In most examples of apples factors other than scab may lower the grade of the apples. Therefore to assess the loss in value due to scab it is necessary to go over the apples twice. On the first occasion the scab blemishes are ignored and the apples put into the correct grades considering size, colour, insect and weather damage. The apples with scab are then placed into their appropriate grades. By subtracting the second figure from the first the change in quantity in each grade due to scab can then be calculated. From this can be obtained the loss in value of crop. It must be emphasized that the figures obtained will apply only to the variety sampled in that season.

Apple brown rot presents a different problem, as here it is not loss of quality but actual loss of fruit which has to be assessed. The difficulty that arises is that the loss takes place over a long period on the trees and also in boxes after picking. To obtain precise estimates of loss we found it necessary to make weekly counts on marked branches (Croxall, Collingwood & Jenkins, 1951). Using the trees examined in these experiments we have attempted to draw up a word picture key for use in rapid survey work.

Brown rot of apples

- | | |
|-----|--|
| % | |
| 0 | No infected fruit seen on tree |
| 0.1 | Single isolated infected fruit on tree |
| 1 | Occasional isolated infected fruits or trusses seen |
| 5 | At least one infected apple seen on every other main branch |
| 10 | At least one infected apple on every main branch |
| 25 | At least two infected apples on every main branch. Some fruit fallen on ground |
| 50 | Every third apple or truss of apples infected |
| | Considerable number of fallen apples |
| 75 | Few intact fruit. Much of crop fallen |

Note

Where possible also make the following records on random samples of apples before grading:

- (a) Percentage of apples infected with brown rot.
- (b) Percentage of apples with skin broken. If possible the cause of injury should be recorded in order to obtain information on the precursors of brown rot.
- (c) Number of days between picking and sampling (or, in the case of fruit put into store, total number of days in which fruit has been out of store since picking). This information is important, as the amount of brown rot will depend upon the length of time and the number of fresh holes.

Unfortunately we have had no opportunity since for testing out this key under a variety of conditions.

The aim of these word picture keys is not to replace the more accurate methods used in experimental work but to use them as a supplement. They should also be useful for rapid survey work over a number of orchards in different seasons and enable assessments by different workers to be compared. This is not possible if one relies upon estimates expressed in terms of slight, moderate, or severe without any exact definition of what the individual will place in these classes.

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ASSESSMENT OF DISEASE INCIDENCE IN THE SUGAR-BEET CROP

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Sugar-beet crops are grown under contracts made between the farmer and the British Sugar Corporation, who have an agricultural organization and field staff adequate to supervise all problems concerned with the growing crop. It is of interest to the Sugar Corporation to know the incidence of diseases of economic importance, and their field staff, who see crops almost daily, are obviously the people to get such records.

In 1942 the fieldmen of four factories made quantitative disease reports in place of the general descriptions and sporadic reports about the occurrence of diseases which had been received previously. In 1946 this arrangement was extended to all sugar-beet areas in England, Wales and Scotland, and has been continued ever since. The fieldmen were not expected to embark on the rather specialized task of disease diagnosis and counting merely on receipt of a sheet of printed instructions. First the agriculturists, to whom the fieldmen are responsible, were given an intensive field course in recognizing, counting and assessing the common diseases, and they trained the fieldmen, most of whom have subsequently been to Dunholme for a similar course. Summaries of the disease reports are circulated regularly to those taking part in the survey, and, in conjunction with occasional lectures help to maintain a reasonable average standard of reliability of the reports. Many recent recruits to the field staff have agricultural degrees or diplomas and have an adequate scientific background to undertake such work.

Different methods of assessment are appropriate for different diseases. The relative economic importance of the disease must to some extent decide the amount of attention it receives. The aim in designing methods has been to fulfil certain needs which are best described by quoting K. Starr Chester's monograph (1950) on the appraisal and interpretation of disease losses—'To be most useful, methods should be concerned with losses, not merely disease intensities and they should be comprehensive, complete, accurate to a practical degree, comparable and objective.' I must add to this list of desiderata 'simplicity', which

is essential. Methods must be simple enough to be carried out in the same way each year by 150 people with no special training in plant pathology, without taking an embarrassing amount of their time, or being so laborious as to kill enthusiasm.

The causes of seedling troubles are often difficult to diagnose. Losses develop over only a short period and field diagnosis is uncertain unless the field is visited at a critical time. Often the fieldman's first knowledge of trouble is when the farmer reports that his crop has come and gone. It is usually possible to decide whether losses are due to soil acidity, infection with parasitic fungi, insect injury, strangles or adverse weather. At the end of June fieldmen are asked to report the acreage of sugar beet which has been lost or resown, and to estimate the acreage on which the stand of plants has been reduced below 75 %, as a result of each of these troubles. Each year the acreage reported lost or with reduced stand from black leg and strangles amounts at most to a few hundred; that lost from soil acidity to a few hundred but that with reduced stand to a few thousand. Losses from frost and wind damage are seasonal and in some years may amount to as much as 5000–6000 acres lost or resown. It is clear that seedling diseases do not result in serious losses with present methods of cultivation.

Acreage estimates are also obtained for the incidence of diseases caused by deficiency of trace elements. These are recorded in the categories of low (up to 20 %), moderate (21–60 %) and high (61–100 %) proportions of the plants showing symptoms. These data are collected each month and the reports so arranged that any acreage becoming more severely affected can be transferred into a higher category. The result is expressed at the end of the year as total acreage in the various categories. Less than 400 acres are severely affected by boron deficiency, but the area lightly affected varies seasonally from 700 to 8000. Manganese deficiency is reported in the high (61–100 %) category on up to 1000 acres, whilst between 3000 and 12,000 are lightly affected. Reports of magnesium deficiency tend to be high in years of light virus yellows attack. For instance, in 1951, 12,094 acres were reported in the low category, 2212 in the middle and 1044 in the heavy intensity category, whereas the more usual levels are 3000–7000 for the low, 500–1500 for the middle and 400–800 for the heavy category. These data on deficiency diseases are of value in assessing their importance in various areas, and also to indicate any tendency for their incidence to vary.

Root diseases have been surveyed by examining the washed samples of roots that are taken from each load of sugar beet arriving at the factories for dirt tare and sugar-content determinations. The scheme was carried out for a few years at those factories which could be visited regularly during the beet campaign, so that all roots picked from the samples as diseased could be examined. The tarehouse superintendents were provided with a brochure illustrating and describing eighteen types of root disease which they were likely to encounter, and some became able to diagnose accurately. At first infected roots were picked from all samples; later only from the samples going through on one or two days each week. Violet root rot was the commonest root disease, and up to 5 % of samples contained infected roots. Crown rot—a usually superficial lesion caused by *Phoma betae*, was also common, as were scab, scurfy root (soil acidity) and dry rot (boron deficiency). Other interesting diseases were found, such as eelworm canker, internal fleck, lenticel rot and black wood-vessel disease, which had not been previously detected in the field. The increase in storage troubles, such as clamp rot, became apparent in the samples examined after the end of December. This survey has now been discontinued but could readily be undertaken again should it be desirable.

The incidence of the common diseases showing on the foliage is assessed by counting plants in sample fields. Each of the fieldmen, whose areas contain between 1000 and 4000 acres of sugar beet, counts diseased plants at the end of each month from May to September in one field of about 10 acres. The number of fields sampled each year varies between 135 and 155. The distribution of the sample fields roughly corresponds with the density of the sugar-beet crop in the various parts of the country. As far as possible a field is chosen each year belonging to the same farmer, chosen because he is a consistent grower and his crops

are judged to be representative of the area. The number of plants showing symptoms of various diseases is counted in ten samples of 100 plants situated at equal intervals along the diagonals of the field. Only two diseases thus counted, downy mildew and virus yellows, are of economic importance.

Downy mildew produces distinctive symptoms at first and infected plants are then easily counted. After a few weeks, plants recover and produce fresh leaves. Although the infection may be chronic, diseased plants are then less readily recognized. Only plants showing actively sporing lesions are counted in the survey. Severe attacks of the disease generally occur near seed crops. In 1943 and 1945 there were quite severe outbreaks which got progressively worse during the season and the mean number of infected plants in sample fields in the factory areas where seed crops are grown rose to nearly 6%. A very severe winter followed by the dry summer of 1947 checked the disease. Since then it has never again been severe, the mean infection in the factory areas in the seed crop districts only once reaching 1%.

Most attention has been given to recording the incidence of virus yellows, the disease of greatest importance in sugar-beet crops. The number of plants showing symptoms is counted in the sample fields each month. The loss of yield is directly proportional to the length of time a plant is infected, thus periodical counts of the number of infected plants in a crop will give a measure of its severity.

Counts of aphids are made by examining the last two plants of each 100. In the years of heavy yellows incidence, 1945, 1949 and 1952, infestations of *Myzus persicae* were also heavy. There is no obvious relationship between the infestation of *Aphis fabae* and incidence of virus yellows. A severe limitation to the value of aphid counts at monthly intervals is that peak infestations may easily be missed, giving a false impression of the relative infestations in different years.

The sample fields amount to an area of about 1500 acres, which is a very small sample of the whole crops of 400,000 acres. To check the accuracy of the results, an independent estimate is made of the intensity of infection at the end of August. The factory agriculturists assess, through the reports of the fieldmen and their own observations, the acreage that is infected to varying degree. The four categories used are (a) less than 1%, (b) 1-20%, (c) 21-60%, (d) 61-100%. From these data the mean percentage infection is calculated and agrees reasonably well with the results obtained from the sample field counts.

The results of the surveys for the period 1942-52, recording the incidence of yellows and estimates of the yield losses, have recently been published (Hull, 1953).

No regular observations have been made on the incidence of diseases in sugar-beet seed crops until 1951. Under the existing scheme for certifying sugar-beet stecklings, sample plots of all commercial steckling beds are planted each year at Dunholme for observation, an ideal method to assess those diseases contracted in the steckling bed. For the two seasons during which the scheme has been in operation, seed crops have been remarkably healthy, the mean virus yellows infection in the crops for harvesting in both 1951 and 1952 being only 0.7%. This is, of course, partly because of control measures now applied to the steckling beds, and these plots are laid down with the intention of assessing also the efficacy of the various methods of control.

Another factor of interest in the epidemiology of virus yellows is the distribution of stored mangolds and fodder beets on farms and the extent to which they are infested with aphids. At the end of March and of April the Sugar Corporation's fieldmen survey about twenty holdings around the site of the sample beet field in which they propose making the monthly disease counts. They record the quantity of stored roots and examine twenty roots from each clamp to assess the proportion infested with aphids. From these data a comparison can be made from year to year of the carry-over of aphids in clamps.

The data obtained from these surveys give much scope for exploring the relative importance of factors concerned in the epidemiology of virus yellows. To what extent is the incidence of yellows related to the density of cropping with *Beta* species, with clamp aphid infestation,

with the proximity of seed crops and with aphid infestations? How is it influenced by the weather, both during the preceding autumn, the winter and the current year? Much work remains to be done on these subjects and the surveys are gathering together the necessary data. For this they have their long-term value but we must not too readily draw conclusions from them as to cause and effect. The results are most important as indications of the difficult problems of epidemiology of diseases suitable for study by experiments.

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THE ASSESSMENT OF INJURY BY SEEDLING PESTS OF SUGAR BEET

By F. G. W. JONES, M.A., *School of Agriculture, University of Cambridge*

An outstanding feature of the sugar-beet crop is the possession of a delicate seedling, susceptible to injury by pests from germination, through the singling stage until the development of seven or eight foliage leaves. Seedling pests fall into three groups: those like the wireworm and millepede that feed below ground only and cause loss of stand; those like the mangold fly, the leaf mining of which amounts to defoliation without loss of stand; and the remaining pests such as cutworms, leatherjackets, pygmy mangold beetle, beet carrion beetle, mangold flea beetle and sand weevil, which cause defoliation in varying degrees accompanied by loss of stand due to destruction of the growing point or the severance of the hypocotyl.

The injury caused by these pests may be assessed in terms of financial loss, but this has the disadvantage that costs and prices are constantly changing. Therefore, it is better to express the loss in terms of yield, or, occasionally, in terms of plant population.

Under normal conditions an excess of 'seed' of high germinating power is sown. Less than a quarter of the potential number of seedlings appears above ground and a further small fraction dies before singling. Estimation of the part played by pests in this heavy loss was not possible until the appearance of BHC as an effective soil insecticide. When this is applied in normal fields where there is no special pest problem, either broadcast or as a seed dressing, the increase in plant population is disappointingly small. The results of some twenty field trials may be expressed in round figures as follows: expected number of seedlings 100, losses due to insects 5, losses due to fungi 10, losses due to other causes, mainly physical 60, seedlings surviving 25. The relative loss from pest attacks is reduced still further by the process of singling. Thus, the cost of insecticidal dressings used on sugar beet must be regarded in most cases as an insurance against the occurrence of unexpected attacks, especially where, following recent trends, reduced seed rates and processed seed are used to ease the labour problem of singling.

The assessment of the economic loss arising from defoliation and reduction of stand may be attempted by using insecticide to control the insect responsible and estimating the increase in yield. This is more difficult to achieve in practice than may appear at first sight. Many seedling pests are sporadic in their occurrence, and outbreaks are often not notified until crop injury has already occurred. Moreover, bad weather may hold up the application of insecticide, and the distance to trial sites may be excessive. Worse still, the insecticide used may have effects quite different from those intended. In a field trial for the control of mangold fly at Massingham, Norfolk, in 1949, various dusts and sprays were employed. Plots successfully treated showed very considerable improvement in growth compared with

controls. On harvesting, it was astonishing to find that the yield from certain dusted plots, where treatment was only partly successful, was greater than that from those where mangold fly had been successfully controlled. The dusts, which were used at rather heavy rates, may have had a fertilizer effect on the soil or have killed or repelled aphids, especially those transmitting virus yellows, but none of these explanations appears probable.

This disappointment led to an attempt to assess the effects of defoliation artificially; men with scissors replacing insects with mandibles. Three such trials were attempted in 1951 in which defoliation in varying degrees was effected at the four- and eight-leaf stage. For comparison, the plant population was also reduced in a regular manner by an eighth, a quarter and a half, and certain plots were completely redrilled. The results obtained on harvesting were closely similar at all three trials. Defoliation up to 50 % had no significant effect on yield and only the worst treatments, complete defoliation and redrilling, seriously reduced yield, the reduction being 27 % for 100 % defoliation at the four-leaf stage, 30 % for 100 % defoliation at the eight-leaf stage and 50 % for redrilling at the four-leaf stage. Two further trials were laid down in 1952, when more drastic treatments were applied. A reduction in yield of the order of 10–15 % was produced by 75 % defoliation and of 20–30 % by 100 % defoliation, whereas removal of a half and three-quarters of the plant population, and redrilling, produced losses in yield of 12 %, 45 % and 55 % respectively. In all trials, the treatments had little effect upon the sugar content of the beet, the main action being to modify the size of the plants. The results of the experiments show clearly that sugar beet is capable of withstanding considerable injury before the yield is seriously affected, and that redrilling is to be avoided so long as even half the desired plant population remains, provided it is fairly evenly distributed over the ground. For attacks of moderate severity, the use of insecticides is likely to be uneconomical if the total cost of application exceeds £3 to £5 per acre, bearing in mind that one ton of washed beet is equivalent to 10 % of an average crop and is worth £5 at current prices.

In conclusion, it must be emphasized that defoliation caused by insects is quite different from the artificial defoliation herein described, and it is difficult to decide which is likely to be most harmful to the beet plant. The loss of stand, however, produced artificially in a regular manner, gives a minimum loss because it allows maximum compensation and does not give rise to large areas entirely devoid of plants. Further experiments are necessary in which artificial defoliation and loss of plant are produced in a manner more similar to that caused by pests.

THE ASSESSMENT OF DAMAGE CAUSED TO SOME BRASSICA CROPS BY THE CABBAGE-ROOT FLY

BY D. W. WRIGHT

National Vegetable Research Station, Wellesbourne, Warwick

The damage caused by the cabbage-root fly results from the feeding of the larvae on the root systems of the plant, although occasionally the aerial parts are also attacked. On the roots, the larvae feed chiefly on the cortex, and when numerous they may completely destroy it, both in the main tap root and in the lateral roots. Heavily attacked plants fail to grow normally, they wilt readily and become heavily waxed and either die or make very poor growth. Severe infestations, which kill the majority of plants in a crop, occasionally occur, but more frequently only a few plants are killed and the remainder reach maturity. Examination of the root systems in such crops has shown that a high proportion of the surviving plants have also been attacked. Such infestations adversely affect the growth of the plant, since it can be shown that, when they are controlled, the crop yield is usually substantially improved.

Methods of assessment of cabbage-root fly damage which are based on the numbers of plants killed or obviously stunted in growth greatly underestimate the losses caused. There

are two main methods which may be considered for assessing the damage caused by cabbage-root fly. The first is based on the proportion of the surface of the root system which has been damaged by the larvae. In this method, it is convenient to take into account only the damage occurring on the tap root, for, if side roots also are considered, the problem of recovery of the whole root system from the soil and the assessment of damage on this becomes extremely complex and laborious. In addition, the variation in the amount of damage on the side roots appears to be much greater than that on the tap root. This method of assessment gives no direct measure of the yield performance of the plant, but is of considerable value as a means of comparing the levels of damage on plants of similar size and age. This method has been extensively used on plot trials for determining the comparative values of various insecticidal treatments. The data obtained are somewhat more sensitive than yield data for determining differences in performance between various treatments.

In field experiments, with plots of 100 plants each, a random sample of twenty-five root systems per plot gives a good estimate of damage on the plot.

TABLE 1. *Effect of varying the dosage and concentration of a γ -BHC suspension on the control of cabbage-root fly, Wellesbourne, 1952*

Concentration of γ -BHC suspension (%)	Rate of γ -BHC per 4840 plants (oz.)	Rate of application per plant (pints)	Distribution of damage to root systems Percentage of plants with scores of				
			0	1-3	4-6	7-8	9-10
0.005	1.28	$\frac{1}{4}$	60.0	28.8	9.6	1.6	0
0.01	1.28	$\frac{1}{8}$	74.4	19.2	4.6	1.6	0
0.02	1.28	$\frac{1}{16}$	84.0	12.8	1.6	1.6	0
0.005	0.64	$\frac{1}{8}$	19.2	32.0	28.8	17.7	2.4
0.01	0.64	$\frac{1}{16}$	47.7	28.8	19.2	7.3	0
0.005	0.32	$\frac{1}{16}$	9.6	20.8	30.4	28.0	11.2
Untreated	—	—	0	10.4	31.2	35.2	23.2

Undamaged roots—all differences between treatments significant.

The second method of assessing damage caused by cabbage-root fly is to record the yield from two series of plots, on one of which the pest has been controlled. There are many factors in addition to insect pests which affect crop yield, principally soil fertility, weather, cultural conditions, variety and disease. Many of these factors are interdependent: thus, it has been shown (Barker & Tauber, 1951) that pea plants raised with a complete nutrient supply showed much less damage from pea aphid attack than plants suffering from nutrient deficiency. Similarly, Wright (1940) showed that the yield increase in summer cauliflowers resulting from the control of cabbage-root fly may be influenced by the amount of nitrogen available in the soil. Spraying with insecticide increased the crop yield by 110.4%; where a top dressing of nitro-chalk was given, in addition, the increase was 204.4%. Plots which received nitro-chalk only and no insecticide showed no significant improvement in yield over the control plots.

From 1939 to 1951 we carried out, almost every year, replicated plot trials for the control of cabbage-root fly at the Horticultural Research Station, Cambridge. These have given a considerable amount of data on the effect of this pest on crop yield and quality, and on the yearly variation in the incidence of attack. The experimental crop used throughout was summer cauliflower which was transplanted in early April. The data obtained, therefore, relate to the incidence of the first generation of root fly on the same crop and in the same locality. In 1951 and 1952, yield experiments were carried out at the N.V.R.S., Wellesbourne, on summer cabbage and on summer and autumn cauliflowers. Similar trials on summer and autumn cauliflowers were also made in 1952 at the N.A.A.S. Experimental Horticultural Station, Luddington.

Table 2 shows the improvements in yield which resulted from the use of insecticides at the various centres. Very substantial increases followed treatment both at Cambridge and at Luddington. The trials at Wellesbourne, however, showed smaller increases than were obtained on comparable crops at other centres.

TABLE 2. *Improvements in yield of brassica crops following the use of insecticides for the control of cabbage-root fly*

Locality	Year	Crop	Percentage increase in yield following treatment
Cambridge	1939	Summer cauliflower	83.5
Cambridge	1940	Summer cauliflower	110.4
Cambridge	1945	Summer cauliflower	49.4
Cambridge	1951	Summer cauliflower	46.5
Wellesbourne	1951	Summer cabbage	22.7
Luddington	1952	Summer cauliflower	41.2
Luddington	1952	Autumn cauliflower	51.6
Wellesbourne	1952	Autumn cauliflower	10.9

The improvements in yield which follow treatment appear almost invariably to be accompanied by significant increases in the proportion of first-grade produce obtained. To be placed in this category, the heads of cauliflowers must reach a certain minimum size (6 in. in diameter) and possess certain desirable quality characteristics such as good colour and texture and an absence of bracts in the curd. There is now considerable evidence to indicate that these quality characters are adversely affected by root-fly attack.

TABLE 3. *Grade composition of the heads from treated and untreated plots of summer cauliflowers, Cambridge*

	1939		1940		1945	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
Percentage of 1st grade heads	71.1	30.6	46.2	17.1	63.9	26.4
Percentage of 2nd grade heads	25.0	57.1	46.2	40.7	27.8	25.0
Percentage of 3rd grade heads	3.3	12.3	7.6	42.2	8.3	48.6
Percentage increase in number of 1st grade heads compared with untreated plots	184.4	—	229.1	—	142.1	—

Table 3 shows the grade composition of the heads from treated and untreated plots of summer cauliflowers in three trials at Cambridge.

The infestation in any of these years was not severe since less than 15 % of the plants on the untreated plots were killed. However, very considerable improvements in the yield and quality of the crops resulted and these reflect the damage caused by the root fly.

In the three trials carried out with cauliflowers at Wellesbourne and Luddington in 1952, significant increases in the number of first-grade heads followed treatment in all cases.

Relationship between rainfall, root infestation and the increase in yield following calomel treatment

Table 4 shows the levels of root fly infestation on treated and untreated summer cauliflowers in randomized block experiments carried out at Cambridge over the period 1939–51. Where yield data were obtained, the comparative increases resulting from treatment are also given. The table gives the rainfall for May and June of each year, and the average monthly rainfall for these 2 months.

TABLE 4. *Relationship between rainfall, increase in yield following calomel dust treatment, and severity of root infestation of summer cauliflowers, Cambridge 1939-51*

Year	Rainfall		Percentage increase in yield following use of calomel dust	Percentage of roots severely infested	
	May	June		Calomel treated	Untreated
1939	1.68	1.57	62.2, trial I	4.4	85.6
1939	1.68	1.57	83.5, trial II	3.0	95.0
1940	1.98	0.43	110.4	3.0	94.0
1941	1.73	1.02	—	2.7	81.3
1942	2.10	0.46	—	0.6	79.2
1943	1.71	1.93	—	—	—
1944	0.94	1.25	—	—	—
1945	1.95	1.63	49.4	4.2	73.6
1946	2.06	3.05	—	—	—
1947	0.35	2.89	—	—	—
1948	3.10	3.02	1.1	1.2	63.3
1949	2.55	1.25	88.5	8.4	100.0
1950	3.13	1.25	—	0	58.3
1951	2.52	1.31	46.5	1.7	90.0
1939-51 average	1.98	1.62	—	—	—

After the crop had been harvested, the root systems were examined and graded according to the degree of root-fly damage, using a standard system of grading from year to year. The natural incidence of attack, as measured by the severity of damage on the untreated crop, shows considerable yearly variation. These fluctuations do not appear to be correlated with the amount of rainfall in May and June, the main period of infestation and crop growth (cf. 1949 and 1950). The extent of the increase in crop yield resulting from the use of calomel dust, however, shows a significant negative correlation (-0.87) with the rainfall in these months. Thus, in 1940 and 1949, following a low rainfall in June, considerable crop increases were recorded.

Very large differences in vigour and uniformity between the plants on treated and untreated plots were observed in 1941, 1942 and 1947, the rainfall in either May or June of each year being below average. When the rainfall in both months was near the average, however, as in 1939, 1945 and 1951, substantial crop increase resulted from treatment. In 1946 and 1948, when the rainfall in both May and June was well above normal, there was no visible or significant improvement following treatment—growth in all cases being equally vigorous.

RELATIONSHIP BETWEEN ROOT INFESTATION AND YIELD IN PRIMO CABBAGE

It has been shown that the effect of a given level of cabbage-root fly infestation on crop yield varies from year to year. The difference in effect appears to be related to the amount of available water in the soil, whilst factors affecting soil fertility also appear to be prominent in determining the extent of crop improvement which may follow root-fly control. It is not possible, therefore, to relate the reductions in yield in different crops from assessments of damage made on their root systems. Where environmental conditions are more nearly uniform, however, as in a field trial, then the yields from the various plots appear to be related to the levels of root infestation on these plots.

Table 5 shows the relation between root infestation and yield in an experiment carried out on summer cabbage at Wellesbourne in 1951. Treatments 1 and 7, assessed either in relation to yield or to uninfested or severely infested root systems, gave performances significantly

TABLE 5. Relationship between yield and root infestation in summer cabbage, Wellesbourne, 1951

Treatment	Rate of application per 4840 plants	Yield (tons/acre)	Percentage of root systems	
			Uninfested	Heavily infested
1. 4 % calomel dust	45 lb.	12.5	31.5	33.0
2. 0.17 % γ -BHC dust	45 lb.	14.4	90.5	3.0
3. 0.45 % γ -BHC dust	17 lb.	13.8	94.0	1.0
4. 0.45 % γ -BHC dust	34 lb.	14.1	91.5	4.0
5. 0.005 % γ -BHC suspension	$\frac{1}{4}$ pint per plant	13.8	68.5	12.5
6. 0.01 % γ -BHC suspension	$\frac{1}{8}$ pint per plant	13.6	82.0	6.0
7. Untreated	—	11.5	1.5	79.0

Significant differences between treatments at the 5 % level are as follows:

Yield: 7 < all others; 1 < 2 to 6; no differences between 2 to 6.

Uninfested roots: 7 < all others; 1 < 2 to 6; 5 < 2, 3 and 4; 6 < 3; no differences between 2, 3 and 4.

Heavily infested roots: 7 > all others; 1 > 2 to 6; 5 > 2, 3 and 4; 6 > 3; no differences between 2, 3 and 4.

different both from each other and from the remaining treatments. The yield data for the remaining treatments (2 to 6), however, showed no significant differences, but further differences appeared in the root infestation data.

Other cabbage-root fly experiments have also shown that root infestation data are more sensitive than yield data for assessing differences in performance between treatments.

CONCLUSIONS

The assessment of root infestation is a useful method for comparing the incidence of attack on crops of similar age and size. It is especially valuable for determining the variation in natural incidence of attack and the difference in performance between various insecticidal treatments when used in the same trial. It does not appear possible, however, to use the method as a basis for the assessment of crop damage.

The extent of crop damage caused by the root fly varies not only with the incidence of the pest but is affected also by certain environmental conditions, of which soil moisture and soil fertility appear to be prominent. It appears that a full estimate of the damage caused by this insect can only be obtained from yield trials.

Trials carried out over the last 12 years have shown that cabbage-root fly is responsible for very considerable losses in yield, particularly with crops transplanted early in the year. Crops transplanted later and harvested in the autumn or winter are usually less affected, but marked increases in yield may be obtained also with these when the pest is controlled.

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REVIEWS

Field Experimentation with Fruit Trees and other Perennial Plants. By S. C. PEARCE.
Pp. 131. Technical Communication No. 23 of the Commonwealth Bureau of
Horticulture and Plantation Crops, East Malling, Maidstone. 1953. 10s.

Fruit trees are not expendable, and this has important consequences in the design of orchard experiments. The value of the experimental unit is high, and therefore the research worker demands designs with a low long-term risk, enabling the maximum use to be made of auxiliary information and permitting a succession of trials on the same trees. Chapter 1 considers these general characteristics of trials with perennial plants, and contains a short discussion on the uses of statistics in general and the concept of significance in particular. The intermingling of the theoretical and practical is typical of the communication as a whole. Two chapters follow on experimental designs, simple and complex, most stress being laid on the randomized block, Latin square, and simple split-plot designs. (The reviewer is glad to see a warning given against the use of the Graeco-Latin square for interacting sets of treatments, a point often overlooked by writers on experimental design.)

Chapter 4 deals with calibration, i.e. the use of auxiliary measurements, such as trunk girth and previous crop yield, for the assessment of individual tree differences. It includes a description of the 'stripe' and 'tile' designs, developed by the author. The name 'calibration trial' is rather confusing; the layouts proposed consist of several varieties systematically planted in such a way that a number of different possible trials can conveniently be laid down on them in the future. Whether or not the trees are calibrated in the years when no differential treatments are applied is irrelevant to this property. Conversely, a commercial orchard may have auxiliary measurements made before treatments are applied, and this could reasonably be called a 'calibration trial'.

In a chapter on 'Blocks, plots, and replications', the author is rightly suspicious of the claims made from uniformity trial data that there is a 'best' plot-size for a crop, and prefers to restrict himself to general considerations rather than to give hard-and-fast rules.

There is a useful discussion in chapter 6 on the relationship of trials to the subject being investigated. Manurial trials are divided into 'definitive' and 'tentative' types, which belong, roughly, to 'pure' and 'applied' research respectively. The author has some interesting comments on layouts for a sequential type of factorial experimentation in 'tentative' trials, in which treatments are added or subtracted as the need arises.

Many will think Dr Pearce's dictum that 'The method of analysis to be adopted should be uniquely designated at the time the experiment is designed and should not be departed from', to be an ideal rather than a working rule, for it is surely not always possible. Nevertheless, the value of 'nominating' effects to be tested in the analysis is clearly brought out, and the author makes an attack on the vexed question of significant differences among a set of means. The analysis of several years' results and of experiments at several sites is briefly discussed. The condemnation of the use of sites as replicates is rather sweeping. While no one would be likely to use sites in Northumberland and Devon as replicates for a variety trial, a number of small trials on different sites in a fairly homogeneous district may be of great value, especially when the commercial nature of the sites, or the size of the plots, limits the number of plots at each site.

A chapter on mishaps and remedies includes a description of the missing-plot technique, and exact results are given for the first time for variances of comparisons in a Latin square layout with one or two missing plots. The final chapter deals with the taking of records, sampling methods, eye estimation and kindred topics.

In four appendices, the author gives, among other things, a lucid account of the additive

model underlying the analyses of variance and covariance, and describes the estimation of parameters in the general, non-orthogonal, case. The habit of some authors of describing in detail the computations arising from a model which is left to the imagination, has given many biologists the feeling that statisticians pull their analyses out of a hat labelled Higher Esoteric Knowledge. Dr Pearce's clear statement of the bases of the analyses will be valuable in allaying any such suspicion.

The communication is attractively printed, and the text is remarkably free from misprints. The price must be considered most reasonable. Altogether this is a valuable addition to the Technical Communications of the Commonwealth Agricultural Bureaux.

J. A. NELDER

The Comparative Biochemistry of the Carotenoids. By T. W. GOODWIN. Pp. 356. London: Chapman and Hall Ltd. 1952. 50s.

To most biologists and biochemists the term 'carotenoid' signifies little more than a vitamin A precursor, and it comes as a surprise to many to find that the carotenoids are amongst the most widely distributed of naturally occurring groups of pigments throughout the plant and animal kingdoms and that they probably have the most varied of functions. Despite the ubiquity of these particular pigments it is equally surprising to find that no comprehensive treatise on them has been published since 1935; since that time, of course, our knowledge of their distribution, biogenesis and functions throughout the plant and animal kingdoms has been very considerably extended. Dr Goodwin has, therefore, earned the gratitude of the whole biological world for the care and patience with which he has sought to assemble in a comprehensive monograph the exceedingly diverse knowledge of carotenoids scattered throughout the world's scientific literature.

It is not surprising that as a biochemist he has approached his subject with a definitely biochemical bias. This viewpoint needs no apology, for, as he states, the most promising lines for future advances of our knowledge of the subject, whether by the botanist, zoologist, entomologist, micro-biologist or other specialist, will be along those of biochemistry. Nevertheless, the treatment of the subject is not so completely biochemical as to be unintelligible to specialists in other branches of science, and in reading through the book one is at all times conscious that the author has treated his subject on much broader lines than those of pure biochemistry.

After a brief introduction to definitions and accepted nomenclature of the carotenoids, Dr Goodwin discusses the occurrence and functions of these pigments in each appropriate phylum of the plant and animal kingdoms, and, to the reviewer at least, he has contributed a very interesting chapter on the conversion of carotenoids into vitamin A. With deliberate intent he has omitted two important aspects of the subject: (a) the changes undergone by the carotenoids on storage and in food storage, which he feels more properly belong to the field of food technology; and (b) the carotene requirements of different species of animals, a subject more suited, perhaps, to a monograph on vitamin A. It is a matter of opinion whether the omission of these subjects altogether, if only in very condensed form, does not somewhat detract from the value of the book, and possibly in a future edition the author might reconsider his decision.

In the concluding chapter Dr Goodwin attempts to make a general assessment of the significance of the very considerable knowledge of the carotenoids now available, and emphasizes the paucity of our knowledge of their information and function. He stresses that the time seems propitious for investigations aiming at the integration of carotenoid biochemistry into a sound and comprehensive theory of carotenoid metabolism. There is no doubt that by his efforts to summarize existing knowledge in such an intelligible form Dr Goodwin has at least marshalled all the important facts for the next stage of such investigations, and by so doing he has rendered a valuable service to the biological and

biochemical worlds. The book is a mine of information, all of which has carefully been sorted and its value assessed.

One final comment is that although we live in times when money seems to have lost much of its customary value the price of this book may prove a serious deterrent to many who would undoubtedly profit greatly by its purchase.

A. EDEN

Cytochemistry. By J. F. DANIELLI. Pp. 139. New York: John Wiley and Sons Inc. 1953. 32s.

In the opinion of the author of this book the preparation of a text-book of Cytochemistry at the present time would be premature. This small monograph makes no such pretensions. It is in fact a slightly enlarged version of a series of lectures delivered by Prof. Danielli in Chicago in 1949. It appears in a Series the purpose of which is to permit an author to write in a somewhat more expansive style about his own work and that of his immediate associates than he can do in ordinary scientific papers. Looked at from that point of view this is a very readable and stimulating little book. The main burden of the tale is the necessity for viewing each step in every cytochemical procedure with the greatest degree of scepticism. The test for alkaline phosphatase figures extensively by way of illustration and it has survived the critical scrutiny of the author and his colleagues (though it is worth noting that a further scrutiny, of more recent date, by Linderström-Lang and Holter, has thrown renewed doubts upon the accuracy of the localization of this enzyme—which only shows how right is the author of this book). Prof. Danielli combines his highly critical approach in matters of practical detail with a fine flair for speculation: on the cytochemical mechanism of secretion, on the role of nucleic acids in protein synthesis, on phosphatase as the enzymic centre of contractile proteins, and on the future contributions of cytochemistry to medicine and agriculture. He leaves the impression that while cytochemistry undoubtedly has a great future, it contains at the moment a large element of 'wishful thinking'. The book contains few typographical errors, but for an entomologist it was saddening to observe a reference on p. 71 to 'the tick *Rhodnius*'.

V. B. WIGGLESWORTH

Krankheiten und Schädlinge der Kulturpflanzen und ihre Bekämpfung. By H. BRAUN and E. RIEHM. Seventh edition. Pp. 348 with 290 illustrations in the text. Berlin: Paul Parey. 1953. DM. 26.80.

The sixth edition of 'Braun-Riehm' was published in 1950 and that a seventh edition has been called for after only three years testifies to the popularity and usefulness of this book in Germany. The last two editions were revised by Prof. Braun but, in the new edition, each author has contributed particular sections of the book although this is not obvious in the style. The adoption of slightly smaller type and a slightly larger page has enabled the authors to reduce the length of the book by a few pages, in spite of the incorporation of much new material, 47 new illustrations, and over 200 additional references.

No change has been made in the plan of the book: a condensed but well-constructed and informative General Part (28 pages) is followed by a Special Part containing an account of the diseases and pests of crops arranged in order of cereals, potato, beet, carrot, clover, lupin, soybean, lucerne, serradella, beans and peas, cabbage and cauliflower, tomato, onion, asparagus, cucumber, turnip and swede, flax, poppy, tobacco, hop, fruit, and vine. The last few pages deal summarily with parasites common to many host plants, and with mice, birds, and weather injury. The book closes with a good index. Each crop is prefaced by a useful key to its diseases and pests, based on symptoms. The textual descriptions are admirably clear and terse; the information is up-to-date and its general level is about or, often, a little

above that of our Advisory Leaflets. There are a few line drawings but the great majority of the diseases and pests are illustrated by photographs, many of which are original, most of which are excellent, and some of which are quite superb.

Criticism may, perhaps, be made of the references which, being inserted as footnotes, show some repetition: 655 are in German, whereas only 52 are in English and 17 in other languages. Even for a book intended primarily for German readers this allocation seems a little unbalanced. Moreover, many of the references are not always the best for the purpose, and of those in English many are inaccurate. Further, about one-half the references concern insect pests but the only entomological reference in English is to a 16-year-old semi-popular account of carrot-fly control. In spite of this, however, the book remains probably the best publication of its kind.

The new edition is dedicated to Geheimen Regierungsrat Prof. Dr Otto Appel—Altmeister des deutschen Pflanzenschutzes—who died on 10 November 1952 at the age of 85. Appel was the senior Honorary Member of our Association, having been elected in 1924.

WILLIAM B. BRIERLEY

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OBSERVATIONS ON THE UPTAKE AND TRANSLOCATION OF FIVE ACTINOMYCETE ANTIBIOTICS BY CUCUMBER SEEDLINGS

By D. PRAMER

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(With Plate 5 and 1 Text-figure)

The uptake and translocation of five actinomycete antibiotics by cucumber seedlings has been investigated by qualitative and quantitative techniques.

Chloromycetin and streptomycin were absorbed from nutrient solution by the root system of cucumber seedlings and translocated to the leaves. The streptomycin concentration of leaf tissue increased with time to a level above that in the nutrient solution, whereas the chloromycetin concentration tended to remain constant at a level below that in the nutrient environment. Chloromycetin and streptomycin were identified in the leaf tissue of treated seedlings by paper chromatography.

No convincing evidence of uptake and translocation of aureomycin, neomycin or terramycin was obtained.

The greater part of our present information on the uptake and translocation of antibiotics by higher plants is of an indirect nature. Partial or complete protection of plants from disease has been presented as evidence for absorption and translocation of antibiotics by roots (Blanchard, 1951; Gopalkrishnan & Jump, 1952), stems (Beale & Jones, 1951; Mitchell, Zaumeyer & Anderson, 1952), and leaves (Leben & Fulton, 1952). That suppression of infection is dependent upon many factors and is, in itself, an unreliable measure of uptake and translocation, has been demonstrated by the recent work of Keyworth & Dimond (1952) and Davis & Dimond (1952).

Though limited in number, studies concerned with a direct demonstration of uptake and translocation of antibiotics by higher plants have been recorded. Anderson & Nienow (1947) grew soybeans in nutrient solutions containing streptomycin and demonstrated antibiotic activity in sap expressed from the aerial portions of the plants. In a similar study, Blanchard & Diller (1951) have shown extracts of lima bean seedlings raised in solutions containing aureomycin, at concentrations as high as 1000 $\mu\text{g./ml.}$, to possess little antibiotic activity. Brian, Wright, Stubbs & Way (1951) have studied the uptake and translocation of griseofulvin. Antibiotic activity was demonstrated in leaf extracts of lettuce seedlings raised in solutions containing griseofulvin, and the guttation-fluid of oats grown in both nutrient solution and soil to which the antibiotic had been added. Winter & Willeke (1951*a, b*) have shown antibiotic activity in guttation-fluid and expressed sap of cress seedlings raised in penicillin solutions. Cress grown in streptomycin solutions contained antibiotic activity in expressed sap but not in guttation-fluid.

The present communication describes qualitative and quantitative studies of the uptake and translocation of five actinomycete antibiotics by cucumber seedlings.

EXPERIMENTAL MATERIALS AND GENERAL METHODS

The following antibiotics were used in the experiments described below: aureomycin hydrochloride, chloromycetin, neomycin sulphate, streptomycin sulphate and terramycin hydrochloride. All but the neomycin preparation were crystalline.

Cucumber seeds (var. Bedfordshire Prize Ridge) were germinated on moist filter-paper and raised in a modified Pfeffer solution. When seedlings had reached the first-leaf stage, the nutrient was replaced by similar solutions containing antibiotics. Control plants received normal nutrients only, and all solutions were renewed every 48 hr.

The antibiotics were assayed by the cylinder-plate technique. The test organism in the case of chloromycetin, neomycin and streptomycin was *Bacillus subtilis* (NCTC 7241). A strain of *B. mycoides*, no. 10 in the collection maintained at these laboratories, was used for the assay of aureomycin and terramycin. The assay medium was Lab-Lemco agar.

EXPERIMENTAL METHODS AND RESULTS

General observations on toxicity

In preliminary studies, each antibiotic was tested for phytotoxic effects at concentrations ranging from 10 to 500 $\mu\text{g./ml.}$ At the higher concentrations, root and shoot growth was stunted in every case. Streptomycin produced no other gross phytotoxic effect. Aureomycin and terramycin caused a chlorotic yellowing of the first and subsequent leaves. Exposure to high chloromycetin concentrations resulted in flaccidity of the whole seedling, whereas neomycin caused cotyledons to become flaccid while stems and leaves retained their turgidity. Pl. 5, fig. 1*a*, shows seedlings grown in 50 $\mu\text{g./ml.}$ solutions of each antibiotic to be comparable to control plants, whereas a tenfold increase in antibiotic concentrations is shown to result in severe phytotoxic effects (Pl. 5, fig. 1*b*). Throughout the work described below the antibiotics were used at a concentration of 50 $\mu\text{g./ml.}$

Qualitative studies

Two simple qualitative tests were used to demonstrate uptake and translocation of the antibiotics.

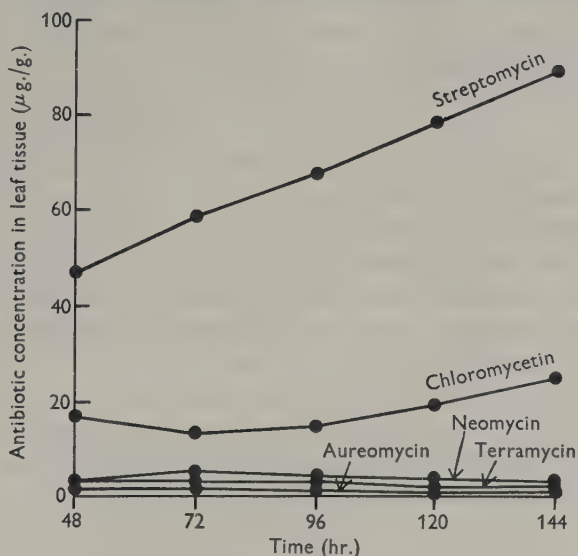
Cucumber seedlings grown in antibiotic-containing nutrient solutions for 5 days were cut across the hypocotyl. Triangular tissue sections, cut from the centre of the cotyledons and leaves, were crushed between glass and then placed on seeded agar plates. The cut stems, with their roots still immersed in antibiotic solution, were allowed to stand at room temperature until a drop of exudate appeared at the site of decapitation. The exudation drops were absorbed by contact with filter-paper disks (6 mm. diameter) and the disks placed on the surface of seeded agar plates.

After 18–24 hr. incubation at 30° C. the plates were examined for zones of inhibition.

The results obtained show the presence of antibiotic activity in both the leaf tissue and cut-stem exudate of seedlings raised in chloromycetin and streptomycin solutions. Typical inhibition zones are shown in Pl. 5, fig. 2. No antibiotic activity was demonstrated in either the leaf tissue or cut-stem exudate of seedlings grown in aureomycin, neomycin or terramycin solutions.

Quantitative studies

A quantitative estimate of the uptake and translocation of each antibiotic was obtained by the following procedure: the cotyledons and leaves of plants growing



Text-fig. 1. Quantitative studies of the uptake and translocation of antibiotics by cucumber seedlings.

in antibiotic-containing nutrient solutions were periodically collected and triturated with a known volume of distilled water. The resulting extracts were centrifuged and the supernatant liquids assayed for antibiotic activity by the cylinder-plate technique. From the weight of tissue extracted, the volume of water used for extraction, and the antibiotic activity of the extract, the antibiotic content of the leaf tissue was calculated. For each determination three seedlings were taken, the leaves of each treated separately, and an average made of the results obtained. Determinations were run 48 hr. after the seedlings were placed in antibiotic solutions and every 24 hr. thereafter for a total of 144 hr. The results are illustrated in Text-fig. 1.

The antibiotic concentration in leaf tissue of seedlings grown in streptomycin solutions was found to increase with time. At the termination of the experiment,

the antibiotic content of the tissue ($90\text{ }\mu\text{g./g.}$) was almost twice that of the solution in which the plants were grown ($50\text{ }\mu\text{g./ml.}$). Leaf tissue of seedlings raised in chloromycetin solutions showed a relatively constant antibiotic content throughout the period of study. An apparent increase, following 96 hr. of growth, was accounted for by the onset of slight wilting and loss of weight by the plants. The highest concentration of antibiotic ($25\text{ }\mu\text{g./g.}$) demonstrated in the leaf tissue of these seedlings was not more than half that of the solutions in which they were grown ($50\text{ }\mu\text{g./ml.}$). When assayed by the cylinder-plate technique, leaf extracts of plants raised in aureomycin, neomycin, or terramycin solution showed little antibiotic activity. Zones obtained were comparable to those produced when similar extracts from control plants were assayed. Although the quantitative data presented for these three antibiotics (Text-fig. 1) are therefore of doubtful significance, it is evident that very little, if any, antibiotic was present in the leaf tissue of seedlings grown in aureomycin, neomycin, or terramycin solutions.

Identification of antibiotics in leaf tissue

Paper chromatography was employed to identify the antibiotic present in leaf tissue of treated seedlings. Filter-paper strips were spotted with leaf extracts and standard antibiotic solutions. These were developed with *p*-toluene-sulphonic acid in wet butanol (Peterson & Reineke, 1950) for 18–24 hr., air dried, and placed on seeded agar plates. After incubation, the location of an antibiotic substance on the paper strip was marked by a clear zone of inhibition. R_F values were calculated from the distances moved by the active substances.

Chromatograms run on leaf extracts from seedlings raised in streptomycin and chloromycetin solutions showed R_F values identical with pure solutions of these antibiotics. Negative results were obtained when leaf extracts from seedlings grown in aureomycin, neomycin, or terramycin solutions were chromatographed.

DISCUSSION

The studies described show that chloromycetin and streptomycin were absorbed from nutrient solution by the root system of cucumber seedlings and translocated to the leaves. No convincing evidence of uptake or translocation of aureomycin, neomycin or terramycin was obtained. It remains to be determined whether failure of an antibiotic to appear in aerial portions of a plant is the result of discrimination in uptake or inactivation following absorption. However, failure to demonstrate an antibiotic in the stem-exudate of decapitated seedlings localizes any discriminatory or inactivating mechanism in the roots.

It is possible that the appearance of antimicrobial activity in aerial portions of a plant grown in antibiotic solution may result from causes other than uptake and translocation. Physiological processes within the plant may be stimulated to produce an antibiotic-like substance, or, on the other hand, an absorbed antibiotic may be metabolized to a chemically different compound showing similar biological activity.

These possibilities were considered in the work of Brian *et al.* (1951) where the characteristic spiral waving of hyphae caused by griseofulvin was used to identify this antibiotic in guttation-fluid and leaf extracts. In the present study paper chromatography was successfully employed to identify chloromycetin and streptomycetin in extracts of leaf tissue.

Though chloromycetin and streptomycin were both demonstrated to be absorbed and translocated, each showed a different pattern of accumulation in the plant. The streptomycin concentration of leaf tissue increased with time to a level above that in the nutrient solution, whereas the chloromycetin concentration tended to remain constant at a level well below that in the nutrient environment. Whether these observed differences are the result of selective uptake and free diffusion phenomena or simple accumulation of a stable substance, as compared with a relatively unstable one, remains for further investigation.

The present study confirms previous reports (Anderson & Nienow, 1947; Winter & Willeke, 1951*b*) of uptake and translocation of streptomycin by higher plants. The results obtained from studies with aureomycin, however, do not confirm those of Blanchard & Diller (1951). This would be expected, as these workers demonstrated only 1.1–7.7 $\mu\text{g.}$ of aureomycin per g. of leaf tissue of seedlings grown in solutions containing 1000 $\mu\text{g./ml.}$ of the antibiotic. In the present work seedlings were raised in solutions containing 50 $\mu\text{g./ml.}$ of aureomycin and, therefore, no detectable amount would be expected in the leaf tissue. Uptake and translocation of chloromycetin, neomycin and terramycin by higher plants has not been subject to previous investigation.

The author is indebted to Miss Joyce Wright and Mr S. H. Crowdy for helpful discussion and to Miss Olga Edwards for technical assistance. Thanks are also due to Mr L. Hewitt of the Photography Section and to the following firms who provided the antibiotics: The Upjohn Co., Chas. Pfizer and Co., Parke, Davis & Co., and Lederle Laboratories Division of the American Cyanamide Co.

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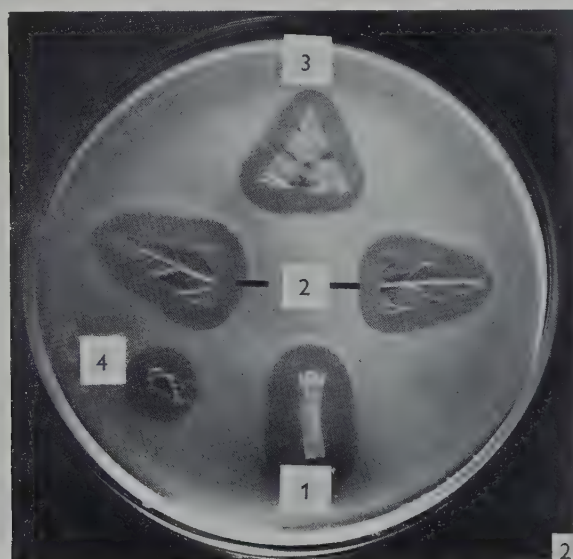
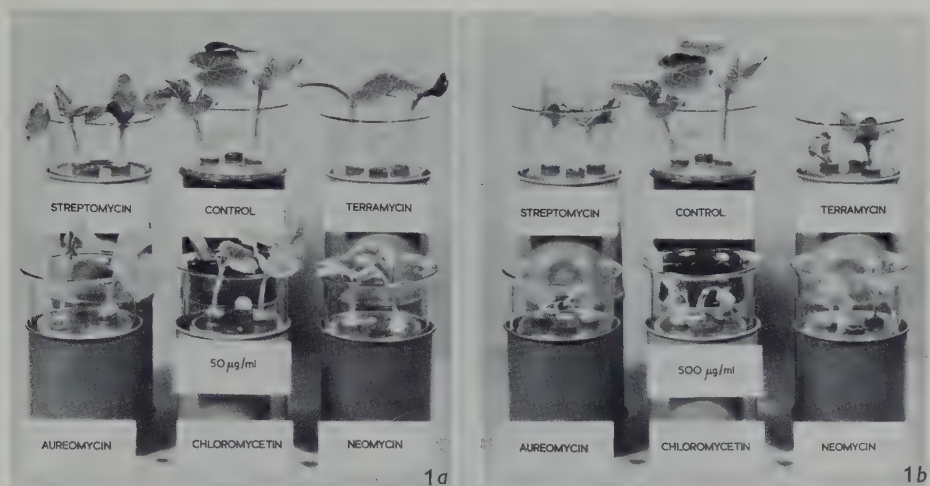
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EXPLANATION OF PLATE 5

- Fig. 1a. The influence of 50 $\mu\text{g./ml.}$ of five actinomycete antibiotics on the growth of cucumber seedlings.
- Fig. 1b. The influence of 500 $\mu\text{g./ml.}$ of five actinomycete antibiotics on the growth of cucumber seedlings.
- Fig. 2. A technique for the qualitative demonstration of uptake and translocation of antibiotics. Cucumber seedlings grown in nutrient solution containing 50 $\mu\text{g./ml.}$ of streptomycin: 1, stem; 2, cotyledons; 3, first leaf; 4, second leaf.

(Received 29 January 1953)



STUDIES IN THE INTERACTIONS BETWEEN SPECIES OF *VERTICILLIUM*

By IVOR ISAAC

University College of Swansea

(With 1 Text-figure)

It is shown that five parasitic species of *Verticillium*, viz. *V. albo-atrum*, *V. Dahliae*, *V. tricornis*, *V. nigrescens* and *V. nubilum*, developed together on agar media or wheat grains as saprophytes without causing mutual antagonism but, when these were injected together, as a mixed spore suspension, into antirrhinum and tomato, suppression of *V. tricornis*, *V. nigrescens* and *V. nubilum* by either one or other of the remaining two invariably resulted. The two most virulent pathogens, *V. albo-atrum* and *V. Dahliae*, were not recoverable from soil after the elapse of 6 months from the time of inoculation; while the other three less virulent pathogens could be isolated after about 12 months in the soil. It is concluded that *V. albo-atrum* and *V. Dahliae* may be described as 'root inhabiting' and *V. nigrescens* and *V. nubilum* as 'soil inhabiting', while *V. tricornis* may be considered as an intermediate type.

It has been shown by the author (Isaac, 1949, 1953) that five parasitic species of *Verticillium*, viz. *V. albo-atrum*, *V. Dahliae*, *V. tricornis*, *V. nigrescens* and *V. nubilum*, differ from each other in their development in culture, and vary in their degree of pathogenic virulence towards a wide range of host plants. In the present work, experiments to investigate the mutual interaction of these species in culture, in host plants and in the soil are described.

In order to test the mutual interaction of these five species when grown on culture media, series of plates of Dox's and potato-dextrose agars and of sterilized moistened wheat grains contained in Petri dishes of 7.5 cm. diameter were inoculated with mycelium of the five species following the two arrangements shown in Fig. 1 *a*, *b*, with the result that the effect of every species upon each one of the other four could be determined. Similar plates of these media were also inoculated with water suspensions of spores of the five separate species, distributed along lines radiating from the centre in the two relatively comparable arrangements shown in Fig. 1 *c*, *d*.

Additional plates of each medium were inoculated with mixed water suspensions of spores of all five species.

On each medium all species developed normally whether growing from mycelium or from spores, showing no apparent mutual antagonism when grown thus saprophytically in close proximity.

To test the interaction of these five species of *Verticillium* upon each other in host plants, seedlings of antirrhinum and tomato about 8–10 weeks old were used, since Isaac has shown (1953) that both *V. nubilum* and *V. tricornis*, although not inducing

wilt symptoms in the former, may nevertheless live parasitically in this host, while the other three species induce disease symptoms in antirrhinum and all five species induce symptoms in tomato. For the purpose of the test, spores from all the fungi were mixed in sterile water, and this mixed suspension was injected by means of a hypodermic syringe into the stems of fifteen of each of the host plants. Further series of three antirrhinum and three tomato plants were inoculated with separate spore suspensions of each of the five species, and these fifteen plants of each host, together with three additional plants of each host injected with water only, served as controls. All the needle wounds were bound with adhesive tape.

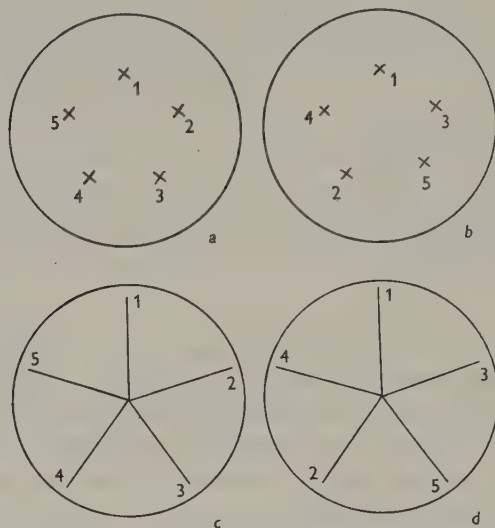


Fig. 1. *a, b*, diagrams to show the relative positions of mycelial inocula on plates of artificial media. *c, d*, diagrams to show the relative positions of radiating lines along which spores were distributed.

Four months after inoculation attempts were made to re-isolate the fungi from all the plants at the wound region and at points on the stem 5–6 in. above. Of the thirty plants inoculated with the mixed spore suspensions, all but eight (three antirrhinum and five tomato) wilted, and no isolate was obtained from any of these eight healthy plants. From the wound region of the remaining twenty-two wilted plants, either *V. albo-atrum* or *V. Dahliae* (but never both) was obtained, together with one or more of the other three species, but from regions of the stems 5–6 in. above the wounds never more than one species was isolated from any one plant, and this species was either *V. albo-atrum* (from eight antirrhinum and seven tomato plants) or *V. Dahliae* (from four antirrhinum and three tomato plants). The presumption that the species present in these regions distal from the wounds were the causal organisms of the wilting seems justifiable.

From the control plants injected with spores of a single species, that species was re-isolated from one, two or all three plants in each series from regions at least 5-6 in. distant from the points of inoculation, thus proving that the spores of all the fungi were viable and capable of germinating to form a spreading mycelium. No isolate was obtained from control plants injected with water only.

All these results indicate that although the five species of *Verticillium* may exist together saprophytically, and appear to grow normally when associated together on agar media and on wheat grains, and, in spite of the fact that each individual one may successfully parasitize antirrhinum and tomato, yet when these hosts are inoculated with all the species together the species *V. tricorpus*, *V. nigrescens* and *V. nubilum* are outgrown by the more virulent pathogens, *V. albo-atrum* or *V. Dahliae*, although the first three species apparently remain alive in the region of the wound, possibly as saprophytes on tissues rendered moribund by the inoculating needle.

To determine the degree of viability and the nature of the interaction between the five species of *Verticillium* in the soil, cultures of these species grown in flasks on sterilized wheat grains for 6-7 weeks were added to soil in pots and in the field respectively as follows:

(a) Every month over a period of 1 year approximately 10 g. of wheat-grain cultures of each species were thoroughly mixed together before being added to John Innes's compost in plant-pots of 10 in. diameter. Eight such pots of soil were so infected each month, while two of uninfected soil were retained as controls in each series of ten pots.

(b) Every month for a year in a field experiment, five drills, approximately 6-8 in. deep and 6 ft. long, were opened and wheat-grain cultures of *Verticillium* were distributed, one species *only* to each drill, along 5 ft. of its length, the terminal foot of each drill being left uninoculated as a control. The drills were then closed with John Innes's compost.

At the end of 12 months, immediately after the last inoculation in each group, attempts were made to re-isolate the fungi, employing the Brierley, Jewson & Brierley (1927) water-dilution method, by taking samples of soil from every pot and from every drill. For purposes of plating 1 c.c. of a 1 : 10,000 or 1 : 20,000 dilution was added to 15 c.c. of medium, and ten plates were poured for each series. The results of these attempts at isolation are shown in Table 1.

Failure to isolate the fungi by this method does not necessarily mean that they were entirely absent from the soil in question, but such failure may be considered a significant indication of their degree of concentration, or their possible existence in the form of some resistant and hitherto unspecified resting structure.

The results summarized in Table 1 indicate that, in both cases, whether the species were mixed before infection or whether they were added separately to the soil, the concentrations of *V. albo-atrum* and *V. Dahliae* rapidly diminished and were not recoverable after 6 months. In contrast, the three other species, *V. tricorpus*, *V. nigrescens* and *V. nubilum*, all of which have been shown to be milder

pathogens than the first two (Isaac, 1949, 1953), were obtainable from the soil 12, 11 and 11 months respectively after inoculation, suggesting that these persist more successfully as soil saprophytes than do either *V. albo-atrum* or *V. Dahliae*. Such variation in the persistence of these species in the soil is of interest since it has been shown above that when they are grown together as saprophytes in the laboratory they all develop normally and persist for an equal period of time. It would appear either that the soil conditions favour the growth of *V. tricornis*, *V. nigrescens* and *V. nubilum* at the expense of the other two, or that these three species are better able to compete with other soil microflora than are *V. albo-atrum* and *V. Dahliae*. The formation in the soil of very resistant resting bodies by the last two which fail to germinate in artificial culture may represent another possible explanation of these findings though one which is difficult to put to the test. This suggestion receives

TABLE 1. *Number of colonies of species of Verticillium isolated from infected soil in pots and in field experiments 12 months after the first inoculation*

Month of inoculation	<i>V. albo-atrum</i>		<i>V. Dahliae</i>		<i>V. tricornis</i>		<i>V. nigrescens</i>		<i>V. nubilum</i>	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field
July	o	o	o	o	o	o	Few	Many	o	o
Aug.	o	o	o	o	o	Few	Many	Many	Few	Few
Sept.	o	o	o	o	Few	Few	Many	Many	o	Few
Oct.	o	o	o	o	o	o	Many	Many	Few	Few
Nov.	o	o	o	o	Few	o	Many	Many	Many	Few
Dec.	o	o	o	o	Few	Many	Many	Many	Many	Many
Jan.	Few	o	Few	Few	Many	Many	Many	Many	Few	Many
Feb.	o	Few	o	Few	Few	Many	Many	Many	Many	Many
Mar.	Few	Few	Few	Few	Many	Many	Many	Many	Many	Many
April	Few	Few	Many	Few	Many	Many	Many	Many	Many	Many
May	Many	Many	Many	Many	Many	Many	Many	Many	Many	Many
June	Many	Many	Many	Many	Many	Many	Many	Many	Many	Many

No species of *Verticillium* was isolated from control soil.

some support from the work of Venkat Ram (1952) who reported a marked stimulation of resistant chlamydospore formation by *Fusarium solani* in the presence of soil bacteria, a phenomenon which he suggested is attributable to the production of antibiotics by the bacteria.

In the attempts at isolation from soil inoculated in June, growth of all species was observed within 2-4 days after plating on agar media, while isolates of *Verticillium albo-atrum* and *V. Dahliae* made from soils infected earlier appeared in greatest number about 7-9 days after plating. Since colonies of these last two species developed from hyaline mycelium and from conidia were normally visible within 2-4 days after inoculation of a plate, it is probable that the isolates obtained from soils infected earlier developed from resting bodies, especially since black 'carbonized' hyphae normally occur in the life cycle of *V. albo-atrum* and microsclerotia in that of *V. Dahliae*. Colonies of *V. tricornis*, *V. nigrescens* and *V. nubilum* appeared in successive crops from 2 to 8 days after plating, a fact which suggests that

these three species were persisting in the soil as hyaline mycelia, conidia or chlamydospores with the additional possibility of microsclerotia and resting mycelium in *V. tricorpus*. These results conform closely to those obtained by Isaac (1946) when series of isolates of *V. Dahliae* were obtained from potting soil in two batches, one 3-4 days and the other 7-8 days after plate inoculation, as a result of which the suggestion was made that the 'delayed' colonies of the second batch probably originated from microsclerotia.

Immediately after the attempts at isolation from soil in June, tomato seedlings 8-10 weeks old were planted in the infected soil, one in each pot and five, 1 ft. apart, in each drill in the field. Controls, one per pot and one per foot of uninfected soil, were also planted.

After 4 months a proportion of the plants were wilted, and both these and those which had not shown disease symptoms were examined to determine the identity

TABLE 2. *Species of Verticillium isolated from tomato plants in pot experiment*

Month of inoculation	No. of plants of in-wilted out of eight in inoculated soil	No. of plants from which fungi were isolated				
		<i>V. albo-atrum</i>	<i>V. Dahliae</i>	<i>V. tricorpus</i>	<i>V. nigrescens</i>	<i>V. nubilum</i>
July	3	3	0	0	0	0
Aug.	2	2	0	0	0	0
Sept.	2	2	0	0	0	0
Oct.	4	3	1	0	0	0
Nov.	3	3	0	0	0	0
Dec.	2	1	1	0	0	0
Jan.	3	2	1	0	0	0
Feb.	4	2	2	0	0	0
March	5	5	0	0	0	0
April	6	5	1	0	0	0
May	5	4	0	1	0	0
June	6	5	1	0	0	0

No species of *Verticillium* was obtained from any control plant.

of any fungus present in the basal region of the stem. In no case was a fungus isolated from any unwilted plant, but all those which had wilted yielded isolates. The results are summarized in Tables 2 and 3.

The results summarized in these three tables indicate that although attempts to isolate *V. albo-atrum* and *V. Dahliae* from the soil 5-6 months after inoculation were unsuccessful, these fungi must have been present since they caused disease of the tomato plants. Furthermore, although the other three species were obtained from nearly all the infected soils, the number of plants from which they were isolated was very few, except for those in soils recently inoculated *separately*, in which the very heavy concentrations of fungal material about the roots of newly planted seedlings presumably increased the chances of infection. It appears from these results that very light concentrations of the more virulent pathogens may be sufficient to cause infection, and that absence of a suitable host probably means that these species must pass into a hibernating condition such as resting mycelia or

microsclerotia, while the other three species may continue an active saprophytic existence in the soil, but in a concentration insufficient to cause appreciable infection. Even where very heavy infection of the soil by these three occurred, as in the pots inoculated with the five species in June just before the seedlings were planted, presence of the two more virulent pathogens apparently prevented *V. tricornis*, *V. nigrescens* and *V. nubilum* from establishing themselves as parasites as they did in the spore-inoculation experiment described earlier.

TABLE 3. Isolations of species of *Verticillium* from tomato plants in field experiment

Month of inoculation	No. of plants infected by each fungus out of a total of five in each series of soil inoculations				
	<i>V. albo-atrum</i>	<i>V. Dahliae</i>	<i>V. tricornis</i>	<i>V. nigrescens</i>	<i>V. nubilum</i>
July	3	0	0	0	0
Aug.	2	2	0	0	0
Sept.	3	1	1	0	0
Oct.	4	3	2	0	0
Nov.	3	2	1	1	0
Dec.	3	4	2	0	0
Jan.	4	4	2	0	1
Feb.	4	4	1	1	2
March	5	4	1	1	0
April	5	3	3	2	0
May	4	5	2	1	2
June	5	5	4	3	2

Two control plants, between drills infected with *V. albo-atrum*, were infected with this fungus, probably due to their roots growing from uninfected soil into the infected region.

CONCLUSIONS

Garrett (1950) suggested that two types of root-infecting fungi may exist in soil, viz. the 'root-inhabiting' and the 'soil-inhabiting' fungi. The former 'are characterized by an expanding parasitic phase on the living host plant and by a declining saprophytic phase after its death', and, 'apart from living host roots, root-inhabiting fungi are distributed through the soil in the form of dead infected host tissue in which the fungi persist as saprophytes and as resting spores and sclerotia'. The soil-inhabiting fungi, Garrett described as 'characterized by ability to survive indefinitely as soil saprophytes', and he stated that 'parasitism is incidental to a saprophytic existence in the soil'.

It would appear, therefore, that *V. albo-atrum* and *V. Dahliae* should be included in the 'root-inhabiting' category, since their apparent concentration in the soil diminished considerably during the year of soil incubation, although remaining sufficient in quantity to cause plant infection at the end of this period. *V. nigrescens* and *V. nubilum* might be placed in the 'soil-inhabiting' class since both are able to survive as saprophytes in the soil in fairly dense concentrations over a relatively long period, and they are certainly only weak pathogens. *V. tricornis* may be considered as intermediate between these two classes, because, although a pathogenic species,

actually much milder than *V. albo-atrum* and *V. Dahliae*, it may nevertheless exist as a saprophyte for a relatively long time in the soil as mycelium and/or conidia. This suggestion, so far as *V. nigrescens* and *V. Dahliae* are concerned, is supported by earlier work (Isaac, 1946) in which the author was able to isolate the former from the soil of the Plant Breeding Institute, University Farm, Cambridge, every month of the year, while attempts to isolate *V. Dahliae* from this soil were unsuccessful except in June, July and August, and even in these months negative results were obtained from soil samples taken 1 ft. or more from sainfoin plants infected with this species.

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THE SPREAD OF DISEASES CAUSED BY SPECIES OF *VERTICILLIUM*

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(With 3 Text-figures)

An investigation has been made of the spread of *Verticillium* disease of tomato and antirrhinum by root contact between diseased and healthy plants, and by the growth of the fungi through the soil. *V. albo-atrum* and *V. Dahliae* spread rapidly by root contact, while *V. tricorpus*, *V. nigrescens* and *V. nubilum* showed no appreciable spread. Where the tomato and antirrhinum hosts wilted and died the causal organism was invariably isolated from the soil near the roots of the dead plants, but where the inoculated host harboured species without showing disease symptoms, as in the case of *V. tricorpus* and *V. nubilum* in antirrhinum, the parasite was never obtained from the soil. Moreover, these species in antirrhinum, and *V. nigrescens* and *V. nubilum* in tomato would appear to be mild pathogens since when they were already well established *V. albo-atrum* was later actually found to enter and parasitize the respective hosts and bring about its own typical disease symptoms.

Spread through the soil in the absence of hosts was tested by the insertion into it of infected wheat grains followed by later attempts to isolate the fungi at varying distances from the point of infection. *V. albo-atrum*, *V. Dahliae* and *V. tricorpus* showed little or no growth through the soil, whereas *V. nigrescens* and *V. nubilum* spread saprophytically to some extent, though never attaining sufficient concentration to cause appreciable infection of later planted tomatoes.

The conclusion is reached that spread of disease caused by species of *Verticillium* takes place mainly by root contact, with a rapidity relatively proportional to that of the death of the host. This finding, together with the lack of spread from antirrhinum plants infected with the non-lethal *V. tricorpus* and *V. nubilum*, suggests that the parasites remain in the vascular tracts until the death of the hosts.

McKay (1926), investigating the *Verticillium* disease of potato in the field, found that spread of infection was more rapid if wilting plants were rogued than if they were left to die, and he regarded this as probably due to the breaking off of fragments of the diseased roots, which decay and so liberate the pathogen into the soil to increase the chances of infection in neighbouring healthy plants. This suggestion was supported by Roberts (1943) who found that spread of *Verticillium* disease of tomato was much more rapid from dead infected plants, the roots of which were probably breaking down and so releasing the fungus into the soil, than from intact living infected plants. Isaac (1946) observed that in the case of wilt of sainfoin in the field caused by *V. Dahliae*, the causal fungus tended to accumulate in the soil in the immediate neighbourhood of the plant first infected, and that the disease appeared to spread from that plant to its neighbours, suggesting that the spread of infection was due either to actual root contact, or to the growth of roots of neigh-

bouring healthy plants into the heavily contaminated soil around the original point of infection. Garrett (1944), discussing the general spread of underground parasites, argued that three classes of such fungi occur—those which spread through the soil and over the underground parts of the host; those which spread only over the underground parts of the host; and those which live inside the vascular system of the host plant. The spread of those in the first category would depend upon an extensive system such as actively growing normal mycelium or rhizomorphs, while in the case of the second and third groups the transmission of the fungus from one host to another must clearly be limited to their points of root contact. He further suggested that the internal parasites do not leave the vascular cylinder of the host until its death is imminent.

In view of these findings and suggestions, an attempt was made to compare the rates of spread by root contact and by growth through the soil of disease caused by five parasitic species of *Verticillium*, which enter the vascular system, viz. *V. albo-atrum*, *V. Dahliae*, *V. tricornis*, *V. nigrescens* and *V. nubilum*.

SPREAD BY ROOT CONTACT

Tomato and biennial antirrhinum plants were selected as hosts because all the pathogens induce disease symptoms and eventually death in the former (Isaac, 1949), while the latter, though capable of parasitization by *V. tricornis* and *V. nubilum* (Isaac, 1953*a*), only wilt and die when infected by any one of the other three, so that the question arises of the possible spread of the first two species from one antirrhinum host to another though inducing no macroscopic signs of their presence.

The plants were grown in boxes approximately 1 yd. square filled to a depth of 6 in. with John Innes's compost, and all inoculations were effected by the insertion of mycelium, taken from Dox's agar medium, into wounds made in the stems just below ground-level. The experiments, which were carried out in a glass-house kept at a moderate temperature throughout the year, were continued through two growing seasons. Throughout this period the many antirrhinum plants which wilted were left in the soil until completely dead before removal for the determination of the causal organism of the disease. The original tomato plants which had died by the end of the first season, either naturally or as the result of infection, were then taken up and the following spring were replaced in precisely the same positions by young seedlings, after which the boxes were 'topped up' with freshly made compost.

All the plants, no matter what their condition when lifted from the soil, were at once examined for the presence of fungi.

Attempts were also made to isolate the various species of *Verticillium* from the respective soils in September of the first year and in January and July of the second year using the Brierley, Jewson & Brierley (1927) water-dilution method, in which, for plating, 1 c.c. of a 1 : 10,000 or 1 : 20,000 dilution was added to 15 c.c. of Dox's agar medium. Chesters's (1940) immersion-tube technique was used initially, but this had to be discarded since the faster-growing soil saprophytes such as *Gliocladium*

roseum, *Mucor* spp., *Pythium* spp. and *Zygorhynchus* spp. grew so quickly over the exposed agar in the test-tubes that no colonies of the slower growing species of *Verticillium* could become established.

Two sets of experiments were carried out with a view to determining: (a) the spread of each separate species; (b) the differential spread when all the species were in competition with each other.

For the former purpose twelve identical series, each consisting of nineteen tomato plants, and twelve similar series of antirrhinum plants were arranged as shown in Fig. 1, and each group was separated into six sets of two series each, five for the purpose of testing the spread of the five separate species, while the remaining set served as a control. The centre plant in each series of the first five sets was inoculated with *V. albo-atrum*, *V. Dahliae*, *V. tricorpus*, *V. nigrescens* and *V. nubilum* respectively. The centre plants of the control series of both tomato and antirrhinum were wounded only.

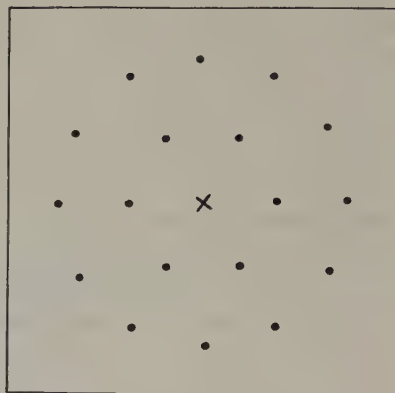


Fig. 1. Diagrams illustrating the arrangement of tomato and antirrhinum plants in each series. Each spot represents the position of one plant; × marks that of the inoculated seedling.

The results of the tomato experiment are shown in Table 1.

TABLE 1. Number of tomato plants from which the different species of *Verticillium* were isolated

Fungus isolated	First season						Second season					
	Series 1			Series 2			Series 1			Series 2		
	Centre plants	Inner circle	Outer circle	Centre plants	Inner circle	Outer circle	Centre plants	Inner circle	Outer circle	Centre plants	Inner circle	Outer circle
<i>V. albo-atrum</i>	1	3	0	1	4	0	1	6	4	1	4	5
<i>V. Dahliae</i>	1	4	0	1	3	0	1	5	2	1	6	2
<i>V. tricorpus</i>	1	0	0	1	0	0	1	1	0	1	0	0
<i>V. nigrescens</i>	1	0	0	1	0	0	1	0	0	1	0	0
<i>V. nubilum</i>	1	0	0	1	0	0	1	0	0	1	0	0

All the plants from which *Verticillium* isolates were obtained had wilted, and no isolate of any description was obtained from any plant in the control series.

It is evident that *V. albo-atrum* and *V. Dahliae* spread quite rapidly from an infected host under conditions of growth which rendered root contact of neighbouring plants possible. On the other hand, *V. tricorpus*, *V. nigrescens* and *V. nubilum* showed little or no spread even after two seasons, despite the fact that by the end of the first season their initial hosts were just as moribund as were those infected with *V. albo-atrum* and *V. Dahliae*.

In September and in July all the pathogens were capable of isolation from the soil near the roots of wilted plants, but not from the vicinity of healthy ones, but in January of the intervening winter, all attempts to isolate *V. albo-atrum* and *V. Dahliae* were unsuccessful, though the other three species were still recoverable.

Somewhat similar results were obtained with the series of antirrhinums. *V. albo-atrum* spread throughout the first and second seasons until thirteen plants in one series and ten in the other developed disease symptoms. *V. Dahliae* also spread until ten and nine plants in the respective series became infected. With *V. nigrescens* the hosts wilted and died, and the species was re-isolated from them, but infection had not spread to neighbouring seedlings. *V. tricornis* and *V. nubilum* did not induce any wilt symptoms, though both were re-isolated from the stem and root regions of the initial host plants, but from no other plants in their respective series.

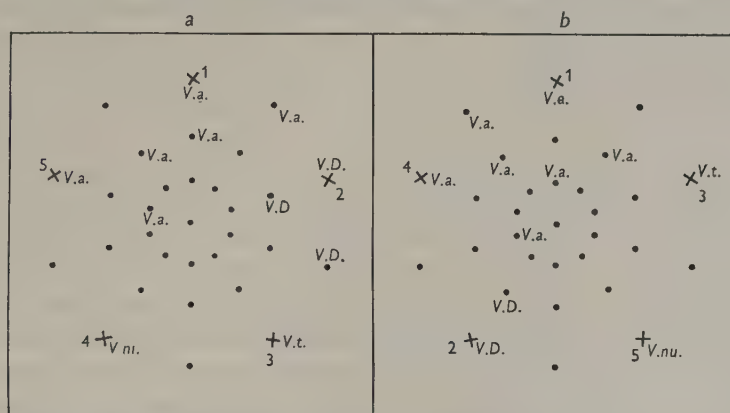


Fig. 2. *a, b*, diagrams to illustrate the arrangement of tomato and antirrhinum plants. A spot marks the position of one plant; each \times shows the place of an inoculated plant; the letters denote the species isolated. *V.a.* = *V. albo-atrum* (1), *V.D.* = *V. Dahliae* (2), *V.t.* = *V. tricornis* (3), *V.ni.* = *V. nigrescens* (4), *V.nu.* = *V. nubilum* (5).

V. albo-atrum, *V. Dahliae* and *V. nigrescens* were all isolated from soil in the neighbourhood of dead antirrhinum plants which they had respectively infected, but all attempts to obtain the other two from the soil of their respective series were unsuccessful, suggesting that they had not passed out into the soil from the still living inoculated hosts.

In order to determine the differential spread when the five species of *Verticillium* were in competition with each other, a planting method on very similar lines to that already described was adopted, thirty-one tomato and thirty-one antirrhinum seedlings being arranged in corresponding series as shown in Fig. 2 *a* and *b*. The plants numbered 1-5 were wound-inoculated with *V. albo-atrum*, *V. Dahliae*, *V. tricornis*, *V. nigrescens* and *V. nubilum*, respectively, the total effect of the two arrangements *a* and *b* resulting in the close proximity of every species to each one of the other four. Ten series were planted, two each of tomato and antirrhinum

inoculated as shown in Fig. 2*a*, two each as in Fig. 2*b*, while the remaining two series served as controls, in which the plants were only wounded and not infected.

During the first season all the inoculated plants and many others in the four boxes of infected tomatoes showed wilt symptoms, and the respective species of *Verticillium* were isolated from these as shown in Fig. 2*a* and *b*, which represent the results in only one series of each duplicate pair, since the two series of each pair corresponded very markedly. *V. albo-atrum* had clearly spread quickly from the seedlings into which it had been inserted and which had died within 5 weeks, to neighbouring plants, and this pathogen was actually isolated from roots of adjacent hosts inoculated with *V. nubilum* (Fig. 2*a*) and *V. nigrescens* (Fig. 2*b*) respectively, while in each case the original inoculum was re-isolated from the stems of the plants, the roots of which had become thus infected. *V. Dahliae* also spread to some extent, while the other three species had not spread at all since they were isolated only from those plants originally inoculated with them. Plants inoculated with *V. Dahliae* and *V. tricornis*, respectively, never yielded any fungus other than the original inoculum, even when these were adjacent to seedlings infected with *V. albo-atrum*, suggesting that these two species are stronger pathogens in tomato than are either *V. nubilum* or *V. nigrescens*, since another and more virulent parasite was shown to enter and develop within such hosts already infected with these last two species.

An examination of plants from which the species were isolated revealed that certain main lateral roots showed no discoloration and gave no isolate on agar, while the xylem of other laterals had become dark brown in colour and contained a species of *Verticillium*. It was noticeable that these latter roots were usually growing towards other wilted plants, suggesting that root contact facilitated spread of the fungus.

Eventually all the surviving tomato and antirrhinum plants were examined for the presence of pathogens with results which are summarized in Table 2.

TABLE 2. Number of 'second year' tomato and of biennial antirrhinum plants infected with species of *Verticillium* at the end of the second season

	Arranged as in Fig. 2 <i>a</i>				Arranged as in Fig. 2 <i>b</i>			
	Series 1		Series 2		Series 1		Series 2	
	Tomato	Antirrhinum	Tomato	Antirrhinum	Tomato	Antirrhinum	Tomato	Antirrhinum
<i>V. albo-atrum</i>	8	7	8	8	7	6	9	5
<i>V. Dahliae</i>	3	5	2	4	4	4	3	3
<i>V. tricornis</i>	1	1	0	1	0	0	1	0
<i>V. nigrescens</i>	0	1	1	1	0	1	0	1
<i>V. nubilum</i>	0	0	0	0	1	1	0	0
No isolate	19	17	20	17	19	19	18	22

None of the plants in the control series was infected, and all the plants from which the species of *Verticillium* were isolated showed wilt symptoms except, as in the experiments recorded above, for the antirrhinum plants infected with *V. tricornis* and *V. nubilum*.

Attempts at isolation of the respective pathogens from the soil yielded results very similar to those described above for the first set of experiments. Where a plant had actually wilted and died the causal organism of the disease was isolated from the vicinity of its roots in September and in July, but in the intervening January, although a few isolates of *V. tricorpus*, *V. nigrescens* and *V. nubilum* were obtained, no colonies of the other two species appeared, and on no occasion were *V. tricorpus* and *V. nubilum* isolated from soil in boxes in which antirrhinums were planted. The soil in the control series yielded no isolate of *Verticillium*.

The soil in all except the control series must evidently have become heavily infected with *V. albo-atrum*, and somewhat less heavily with *V. Dahliae*.

The heavy infection by *V. albo-atrum* was further demonstrated by the fact that those tomato plants parasitized in the second season by this pathogen were scattered indiscriminately throughout the boxes; while, on the other hand, those infected during the second year with *V. tricorpus*, *V. nigrescens* and *V. nubilum* respectively were localized near the situations of the plants originally inoculated with these fungi in the first season, emphasizing the more localized distribution in the soil of these three species.

In the series of antirrhinums *V. albo-atrum* spread rapidly from the inoculated plants during both seasons and even entered both roots and stems of adjacent plants inoculated with *V. tricorpus* and *V. nubilum*, although both of these two latter species were also re-isolated from the wound regions of their respective hosts. *V. Dahliae* also spread to a lesser extent but *V. tricorpus*, *V. nigrescens* and *V. nubilum* were never isolated from any but the plants which had originally been inoculated with them.

The rapid spread of both *V. albo-atrum* and *V. Dahliae* in the tomato series in the first season in both sets of experiments may possibly be accounted for by the early death of the inoculated plants followed, probably as a result of root decay, by a liberation of the pathogens into the soil with a corresponding increase in the possibility of infection of neighbouring healthy roots. The plants inoculated by the other three pathogens survived for a much longer period and decay of their roots in the soil, with the consequent liberation of the pathogens, was therefore probably delayed until a time much nearer that of their natural death at the end of the season and too late for infection of fresh hosts.

The results described above of attempts to isolate the pathogens from the soil, and an earlier report by Isaac (1953*b*) indicate a progressive reduction in the amount of viable material of all five species, but especially of *V. albo-atrum* and *V. Dahliae*, when they exist saprophytically in the soil for several months. Since only slight concentrations of these two virulent pathogens have been shown to be sufficient to cause infection (Isaac, 1953*b*), while disease can only be induced by heavy contamination of the soil by each of the other three, the relatively small amount of *V. albo-atrum* and *V. Dahliae* persisting in the soil at the end of the winter may conceivably account for the widespread disease caused by these pathogens in the tomato seedlings planted in the second season, especially in view of the rapid

growth of the roots of these plants throughout the infected soil. On the other hand, the low incidence of wilt symptoms brought about by the other three species may be accounted for by a decline in the concentration of these species below the minimum necessary to cause infection.

A similar explanation may also account for the marked spread of *V. albo-atrum* and *V. Dahliae*, and the very low incidence of disease caused by *V. nigrescens* in antirrhinum, while the failure of *V. tricorpus* and *V. nubilum* to spread beyond the host plants originally infected is clearly attributable to the inability of these pathogens to kill and therefore cause decay of the roots of the inoculated hosts, with consequent lack of liberation of the fungus into the soil.

SPREAD THROUGH SOIL

The ability of these five species of *Verticillium* to grow through the soil as saprophytes was then investigated. Eighteen boxes, each 2 ft. 6 in. by 2 ft. were filled with John Innes's compost to a depth of 4 in. Embedded in the centre of each in a vertical position was a test-tube 9 in. long, the lower half of which was provided with sixteen holes, of approximately 3–4 mm. diameter, arranged in four vertical rows of four each. The test-tubes were separated into six series of three each, the tubes of each of five series being half filled with wheat-grain cultures of one of the five respective species of *Verticillium* under examination, while uninfected wheat grains, to serve as controls, were placed in the three tubes of the remaining series. The wheat grains were too large to pass through the holes in the tubes though fungal hyphae growing upon them could obviously escape through them into the surrounding soil. The ability of each to do so was therefore tested in triplicate, with triplicate controls. All the boxes were left in a moderately heated glasshouse for 12 months, after which time two glass cylinders, 3½ and 8 in. diameter respectively, were forced concentrically into the soil of each box right down to the wooden base, as shown in plan in Fig. 3. The eighteen test-tubes were then removed with the minimum disturbance to the soil and the remaining holes filled with fresh uninfected compost.

In order to determine whether any spread of the pathogens had occurred attempts were made to isolate the fungi by taking many samples of soil with a narrow cork-borer at varying distances from the points of infection.

The results showed that *V. albo-atrum*, *V. Dahliae* and *V. tricorpus* were dis-

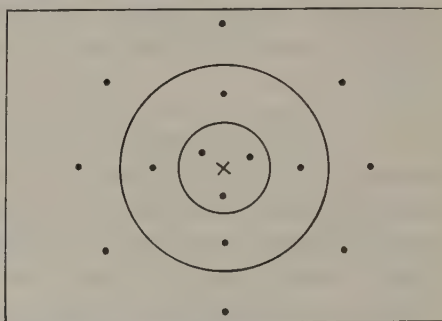


Fig. 3. Diagram illustrating the arrangement of tomato plants. Each spot represents the position of one plant; the two concentric circles indicate the respective positions of the two cylinders; × marks the position of the test-tube.

tributed only very sparsely in the soil, restricted to the immediate neighbourhood of the test-tubes, while *V. nubilum* and *V. nigrescens* could be isolated from soil within radii of approximately $1\frac{1}{2}$ and $2\frac{1}{2}$ in. respectively from the centre of infection. Although failure to isolate the fungi may not necessarily indicate their complete absence from any sample of soil, it may nevertheless be attributable to either a very low concentration or a persistence in a resting condition resistant to germination in artificial culture. No species of *Verticillium* was isolated from the soil in the control boxes, so that pathogens recovered from the soil of the infected ones could have originated only from the infected wheat grains.

After these attempts at isolation a further effort was made to obtain evidence of mycelial spread. In April of the second year, tomato seeds were sown all over the soil in every box and covered by a light dressing of John Innes's compost. The developing seedlings were later thinned out to the number shown in the diagram in Fig. 3. The glass cylinders ensured that the roots of any one ring of plants were not in contact with those of any other ring, so that the wilting of any plant outside the first cylinder would be due not to spread outwards by root contact, but rather to growth of the fungus through the soil from the central source of infection.

After 4 months all the plants were tested for the presence of fungus, and the results indicated that no plant was infected in the soil between the two cylinders or in the outermost zone. The effects on plants within the inner cylinders are shown in Table 3.

TABLE 3. *Number of plants infected by species of Verticillium within the central cylinder*

Fungus	No. of plants infected out of total of 4		
	Box 1	Box 2	Box 3
<i>V. albo-atrum</i>	3	3	2
<i>V. Dahliae</i>	2	3	2
<i>V. tricorpus</i>	1	1	0
<i>V. nigrescens</i>	1	0	0
<i>V. nubilum</i>	0	1	0

None of the control plants was infected. All these results indicate that in one year the species of *Verticillium* spread only slightly from the wheat grains through the John Innes's compost, although this contained as much as 25-30% humus. *V. albo-atrum*, *V. Dahliae* and *V. tricorpus* induced a greater incidence of disease of the central plants than did either *V. nigrescens* or *V. nubilum*, despite the fact that the two last named appear, from isolation tests, to have grown further from the wheat-grain cultures. Moreover, although *V. nigrescens* was isolated from the soil between the two cylinders, not one plant was infected in that region, probably on account of the light infection of this soil. Since the roots of the plants within the central cylinder were in close proximity to each other, root contact may have materially increased the chances of infection by *V. albo-atrum* and *V. Dahliae*; but outside this cylinder, which prevented mutual contact of roots in the two concentric

zones, no infection had occurred, a fact which supports the previous finding that these species are almost completely unable to spread through soil unoccupied by host plants.

CONCLUSIONS

The results of the work reported provide further evidence in support of the author's claim (Isaac, 1953*b*) that while *V. tricornis*, *V. nigrescens* and *V. nubilum* achieve some limited measure of success as soil saprophytes, *V. albo-atrum* and *V. Dahliae* appear to be entirely incapable of spreading as saprophytes through the soil, but remain restricted to the immediate vicinity of the roots of their hosts. Therefore, since these two species are largely responsible for the *Verticillium* wilts of tomato and antirrhinum, the rapid spread of these diseases from an infected plant must be due, not to the growth of fungal mycelium in the soil towards new hosts, but to the growth of the roots of those potential hosts into infected soil around the initially diseased plant. Since such soil infection is accomplished by the liberation of the pathogen on the breakdown of the diseased roots, the more speedy the lethal effect of the pathogen on the host the more widespread will be the incidence of disease. When the parasite is not fatal to the host, as in the case of antirrhinum infected with either *V. tricornis* or *V. nubilum*, no apparent infection of the neighbouring soil results, and therefore no spread of the disease occurs. This appears to support Garrett's (1944) argument that 'a root does not become infectious to other roots in contact with it as soon as it has become infected because the fungus does not emerge from the vascular cylinder until the disease reaches its penultimate phase in the plant'.

The author wishes to express his appreciation of the helpful advice given him by Prof. F. A. Mockridge in the preparation of this paper, and his thanks to Miss K. Woolfenden for her technical assistance.

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OBSERVATIONS ON THE INCIDENCE OF CLUB-ROOT DISEASE OF BRASSICAE IN LIMED SOILS IN RELATION TO TEMPERATURE

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(With 1 Text-figure)

When cabbage plants growing in artificially contaminated, heavily limed soil were maintained at greenhouse temperatures (mean about 23° C.) for periods of up to 12 days and then at shade temperatures in an open-air verandah (mean not exceeding 12.5° C.) for up to 21 days only slight attacks of club root developed. Exposure to greenhouse temperatures for at least 6 days and then in a verandah to a mean air temperature of about 16° C. permitted severe attacks to develop. A very severe attack occurred when plants remained in the greenhouse throughout an experiment, but all the plants were healthy when verandah temperatures only were employed. It is suggested that exposure to favourable temperatures for infection and development of the disease for 12 hr. daily may permit the occurrence of a moderately severe attack.

INTRODUCTION

It has been demonstrated that very severe attacks of cabbage club root caused by *Plasmiodiophora brassicae* Woron. occur in alkaline soils in pots at relatively high temperatures, provided other conditions do not act as limiting factors (Colhoun, 1953). Favourable conditions were provided by a mean air temperature of about 23° C. fluctuating to points lower and much higher than the mean. Lower temperatures were less favourable for the incidence of the disease. In the above work the plants were maintained at the same temperature range throughout the growing period, but in the present experiments the writer attempted to determine the minimum period required at such high temperatures before severe attacks occurred.

The experimental methods previously described were employed. Cabbage plants (variety Enfield Market) were transplanted into artificially contaminated soil, the reaction of which had been adjusted by the addition of calcium hydroxide or calcium oxide. The inoculum for each experiment was obtained from a different source. A constant soil moisture of 70% of the maximum water-holding capacity was maintained by watering on alternate days. As soon as the seedlings were established, the pots were removed from an open-air verandah with a north aspect to a greenhouse, and on the expiration of an appropriate period they were replaced in the verandah. Certain pots were exposed to greenhouse temperatures for a second period of varying duration and afterwards replaced in the verandah. In each experiment some pots serving as controls remained in the verandah or in the greenhouse throughout the growing period.

RESULTS

Experiment 1. In this experiment, the effects of exposing plants continuously to greenhouse temperatures for periods of up to 8 days, followed by verandah temperatures, were compared with those of greenhouse or verandah temperatures throughout the growing period. The mean air temperature during the experimental period was 12.3° C. (range 6–18° C.) in the verandah and 24.8° C. (range 15–39° C.) in the greenhouse. The mean daily temperatures throughout the period for each set of conditions are shown in Fig. 1. The data for soil pH at the end of the experiment, the number of diseased plants and the disease index (Colhoun, 1953) for each pot are stated in Table 1.

TABLE 1. *Effect of varying temperature conditions (Exp. 1)*

(Experimental period 16 April–17 May 1952 (31 days). Soil pH adjusted by use of $\text{Ca}(\text{OH})_2$ to 7.1 at commencement of experiment. Inoculum *ex* cabbage roots. Spore load 2.5×10^7 spores per g. oven-dried soil.)

Period of exposure to greenhouse temperatures (in days)	Soil pH at end of exp.	No. of plants surviving (out of 20)	No. diseased plants	Disease index
0	6.7	20	0	0
1	6.8	20	1	20
2	6.7	20	2	20
3	6.6	20	1	5
4	6.6	20	4	50
5	6.7	20	5	40
6	6.6	20	5	65
7	6.7	20	4	45
8	6.6	20	8	85
Throughout exp.	6.6	20	20	340

It is seen from Table 1 that all the plants maintained in the greenhouse throughout the period were diseased, and the disease index indicates that most of them bore large clubs. None of the plants maintained on the verandah during the entire period was diseased. The results show that exposure of plants to greenhouse temperatures for periods of up to 8 days is inadequate to bring about severe attacks.

Experiment 2. The effects of exposing plants to greenhouse temperatures for longer periods than employed in Exp. 1 were studied in this experiment. Some pots were subjected to greenhouse temperatures for two periods of equal length but separated by an equal period in the verandah. A few pots were exposed to greenhouse temperatures by day (10 a.m. to 10 p.m.) and verandah temperatures by night during the entire experimental period, other pots being kept in the verandah by day and in the greenhouse by night. Certain pots were maintained throughout the experiment in either the verandah or greenhouse. The mean air temperature during the experimental period was 11.6° C. (range 5–18° C.; mean by day 12.8° C.; mean by night 10.1° C.) in the verandah, and 23.1° C. (range 15–40° C.; mean by

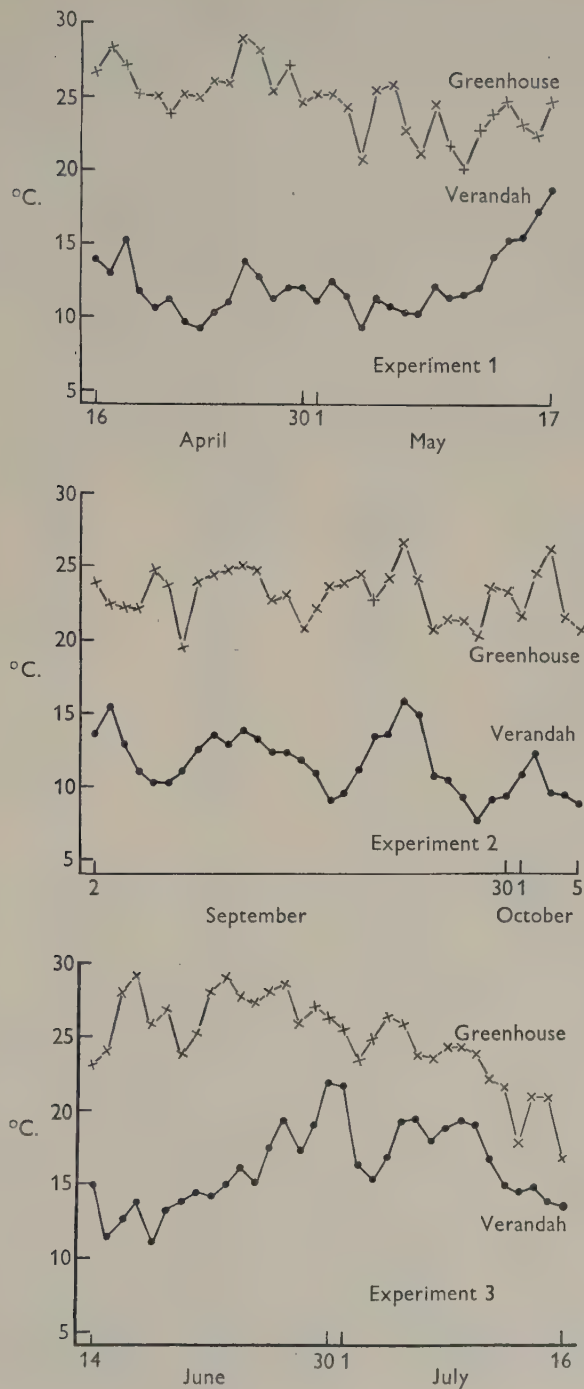


Fig. 1. Mean daily temperatures.

day 26.4° C.; mean by night 19.5° C.) in the greenhouse. The mean daily temperatures for each set of conditions are shown in Fig. 1. The data for soil pH at the end of the experiment, number of diseased plants and disease index for individual pots are presented in Table 2.

The results obtained show agreement with those in Exp. 1 regarding plants maintained throughout the entire period either under greenhouse or verandah temperatures. They also show that exposure to greenhouse temperatures continuously for periods of up to 12 days does not bring about severe attacks. Only slight attacks occurred on plants placed in the greenhouse for two periods of up to

TABLE 2. *Effect of varying temperature conditions (Exp. 2)*

(Experimental period 2 September–5 October 1952 (33 days). Soil pH adjusted by use of CaO to 7.3 at commencement of experiment. Inoculum *ex* cauliflower roots. Spore load 10⁷ spores per g. oven dried soil.)

Growing conditions	Soil pH at end of exp.	No. of plants surviving (out of 20)	No. diseased plants	Disease index
V throughout	6.8	16	0	0
2 days in G then V	6.8	17	0	0
4 days in G then V	6.7	20	0	0
6 days in G then V	6.8	19	4	32
8 days in G then V	6.8	15	3	20
10 days in G then V	6.8	20	7	45
12 days in G then V	6.7	20	11	75
1G+1V+1G* then V	6.8	20	0	0
2G+2V+2G then V	7.0	19	0	0
3G+3V+3G then V	6.8	20	1	5
4G+4V+4G then V	6.8	20	6	35
5G+5V+5G then V	6.8	19	3	16
6G+6V+6G then V	6.9	19	4	42
G by day, V by night	6.7	20	20	180
G by day, V by night	6.7	20	20	215
V by day, G by night	6.5	19	7	37
V by day, G by night	6.7	19	12	100
G throughout	6.8	20	20	385
G throughout	6.5	13	12	286

V=verandah. G=greenhouse.

* 1G+1V+1G=1 day in G then 1 day in V followed by 1 day in G.

6 days each. A moderately severe attack resulted when plants were kept in the greenhouse by day and in the verandah by night, but under the lower temperatures provided by greenhouse conditions at night and verandah conditions by day a less severe attack resulted.

Experiment 3. In this experiment the effects of continuous exposure to greenhouse temperatures for periods of up to 15 days were studied, and as in Exp. 2 some pots were placed in the greenhouse for two equal periods of up to 8 days but separated by a period under verandah temperatures. Some pots were also maintained throughout the period in the greenhouse or verandah. The mean air temperature throughout the period was 16.1° C. (range 8–26° C.) in the verandah,

and 24.8° C. (range 16–39° C.) in the greenhouse. The mean daily air temperatures are recorded in Fig. 1. The values obtained for soil pH at the end of the experiment, number of diseased plants and disease index for each pot are stated in Table 3.

From the results presented it is seen that, as in previous experiments, plants grown in the verandah throughout the period were healthy, while those raised under greenhouse temperatures only were heavily diseased. Under the conditions employed in this experiment, exposure of plants to greenhouse temperatures for 6 days or more was associated with the occurrence of fairly severe attacks, although few plants were diseased when the period in the greenhouse was limited to 3 days. When plants were exposed to greenhouse temperatures for two separate periods,

TABLE 3. *Effect of varying temperature conditions (Exp. 3)*

(Experimental period 14 June–16 July 1952 (32 days). Soil pH adjusted by Ca(OH)_2 to 6.9 at commencement of experiment. Inoculum *ex* cabbage roots. Spore load 2.5×10^7 spores per g. oven-dried soil.)

Growing conditions	Soil pH at end of exp.	No. of plants surviving	No. of diseased plants	Disease index
V throughout	6.7	18	0	0
3 days in G then V	6.7	18	1	11
6 days in G then V	6.7	20	13	170
9 days in G then V	6.7	20	15	175
12 days in G then V	6.8	19	14	221
15 days in G then V	6.7	17	13	206
2G+2V+2G then V	6.7	19	5	74
4G+2V+4G then V	6.6	18	12	195
6G+2V+6G then V	6.7	19	10	132
8G+2V+8G then V	6.7	20	16	190
G throughout	6.8	19	18	263
G throughout	6.7	20	20	305

each of 6 days or longer, the results do not suggest that the second period of exposure was responsible for any substantial increase in the severity of the attack. When each of the two periods in the greenhouse was shorter than 6 days the results would suggest that the second period in the greenhouse increased the intensity of the attack. The most severe attack recorded was on the plants maintained at greenhouse temperatures throughout the experiment.

DISCUSSION

Very slight attacks of the disease resulted in Exps. 1 and 2 when plants were subjected to greenhouse temperatures for 6 days and then placed in the verandah. Even when this period was extended to 12 days in Exp. 2 severe attacks did not occur. In Exp. 3 a relatively severe attack resulted from exposure to greenhouse temperatures for 6 days or more. The temperatures during the 6 days in the greenhouse in Exps. 1 and 3 were very similar although somewhat higher than in Exp. 2. Differences in the intensity of attack in the three experiments cannot be attributed to differences in greenhouse temperatures during this period. Since only

very small differences occurred in the pH values of the soils employed in the three experiments, it is concluded that the heavier attack occurring in Exp. 3 is due to the verandah temperatures being higher than in Exps. 1 and 2. The difference in temperature was most noticeable during the 2-3 weeks following the return of pots to the verandah. This temperature difference may be considered in relation to the observation made in Exp. 2 that a moderate to heavy attack, according to the temperature range employed, followed exposure of plants to greenhouse temperatures for 12 hr. daily.

In these experiments plants in the verandah were subjected only to shade temperatures. It can therefore be appreciated that when plants are grown in the open air, during the summer, in full sun, there is a good chance of suitable temperatures being recorded for adequate periods to permit of attacks occurring in limed soil with a heavy spore load.

The writer wishes to express his thanks to Prof. A. E. Muskett for his continued interest in these studies.

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STUDIES ON THE BACTERIAL DIE-BACK AND CANKER DISEASE OF POPLAR

III. FREEZING IN RELATION TO THE DISEASE

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(With 1 Text-figure)

Artificial freezing caused leaf necrosis and twig die-back but did not produce cracks or cankers on living branches of three varieties of poplar. Freezing increased the damage caused by a well-established infection of the bacterial die-back and canker but checked new lesions. Freezing prior to infection has no appreciable effect on the establishment of the disease.

INTRODUCTION

Day & Peace (1934*a*) and Delevoy & Boudru (1935) suggested that frost played an important part in the bacterial die-back and canker disease of poplar. Lansade (1946) considered that *Pseudomonas syringae* van Hall, together with certain environmental conditions, such as frost, was responsible for the disease. Referring to the failure of Koning (1938), and Ter Pelkwijk & Brink (1946) in Holland, to produce the typical features of the disease by their inoculations, Day (1948) suggested that frost injury predisposed trees to infection by *P. rimaefaciens* Koning, with its accompanying bacteria and fungi.

In the present investigation, the effect of artificial freezing on infection and also on the progress of the disease was studied.

Poplar cuttings a few months old were potted in October 1947 and 1948 and kept in a greenhouse, the temperature of which never dropped below freezing-point during the experimental period. In preparation for the experimental freezing treatments the pots were wrapped in corrugated cardboard, placed in a wooden box (three in each) and surrounded, as shown in Fig. 1, by insulating materials to prevent the soil freezing. The plants were then placed in the freezing chamber and returned to the greenhouse after treatment. Freezing experiments were carried out in the spring, i.e. at the time the plants would be susceptible in the field to both frost damage and to infection with the bacterial disease.

EXTERNAL DAMAGE

Experiment 1

Three poplar varieties were used in 1949. These will be referred to as *Populus serotina* Hartig, *P. eugenei* Simon Louis and *P. trichocarpa* Torr. & Gray. The

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plants were divided into three lots, each of three plants of each variety. The first lot was frozen for 6 hr. on three occasions, viz. 15 March at -10°C ., 13 April at -7°C ., and 16 May at -3.5°C . The second lot was frozen for 6 hr. on 13 April at -7°C . and 16 May at -3.5°C . The third lot was frozen for 6 hr. on 16 May at -3.5°C .

The results (Table 1) and the observations may be summarized as follows:

First lot (plants frozen 3 times): *P. trichocarpa*—young trees were flushing before the first exposure and showed die-back soon after. After the second exposure the new shoots produced below the dead parts were again affected with die-back. The new shoots which were covered with chaff in the second exposure, thus escaping freezing, showed necrotic spots after the third exposure.

P. serotina, though still dormant during the first exposure, was affected with die-back. The plants showed no sign of recovery before the second exposure so no

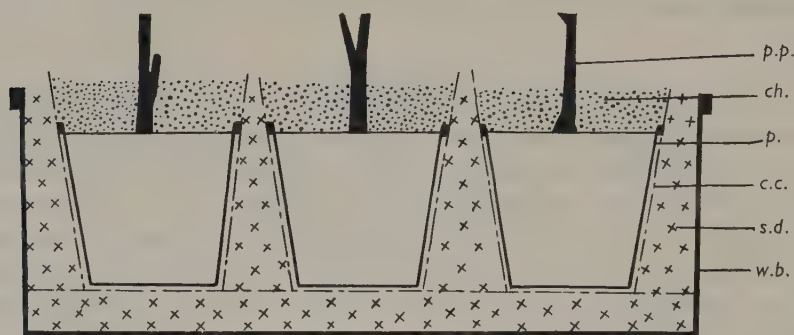


Fig. 1. Diagrammatic representation showing the arrangements made before freezing; *p.p.*, poplar plant; *ch.*, chaff; *p.*, pot; *c.c.*, corrugated cardboard; *s.d.*, sawdust; *w.b.*, wooden box.

new symptoms were observed. After the third exposure the results were as in *P. trichocarpa*.

P. eugenei was dormant during the first exposure and no damage due to freezing was observed. In the second exposure, the plants, which were just flushing, were affected with die-back. After the third exposure, the results were as in the other poplars.

Second lot (plants frozen twice): *P. trichocarpa* and *P. eugenei* behaved after the first and the second exposures as after the second and the third exposures of the previous lot respectively. The leaves and branches of *P. serotina* were killed after the first exposure, but after the second exposure the leaves produced from below the affected twigs showed necrotic spots. Two plants recovered completely during the following summer, and leaves were produced again on all parts of the plants.

Third lot (plants frozen once): all the poplar varieties showed necrotic spots on the leaves, most marked in *P. trichocarpa*.

Experiment 2

Three plants each of *P. trichocarpa*, *P. serotina* and *P. eugenei* were exposed for 6 hr. to $-5^{\circ}\text{C}.$, on 24 April 1950. *P. trichocarpa* was affected with die-back, while *P. serotina* and *P. eugenei* exhibited leaf lesions but the branches were not killed and produced leaves in the following summer.

In Exps. 1 and 2, no cracks, frost cankers or rough bark were observed on the exposed branches of the young trees.

TABLE I. *The effect of freezing on poplars: external damage, Exp. 1**

Lot no.	Variety	Exposure to $-10^{\circ}\text{C}.$ on 15. iii. 49	Exposure to $-7^{\circ}\text{C}.$ on 13. iv. 49	Exposure to $-3.5^{\circ}\text{C}.$ on 16. v. 49
1 (frozen 3 times)	<i>P. trichocarpa</i>	+++	+++	+
	<i>P. serotina</i>	+++	+++	+
	<i>P. eugenei</i>	—	+++	+
2 (frozen twice)	<i>P. trichocarpa</i>	.	+++	+
	<i>P. serotina</i>	.	++	+
	<i>P. eugenei</i>	.	+++	+
3 (frozen once)	<i>P. trichocarpa</i>	.	.	+
	<i>P. serotina</i>	.	.	+
	<i>P. eugenei</i>	.	.	+

* In the table: +++ = leaf lesions and die-back; ++ = leaf lesions and late recovery; + = leaf lesions or necrotic spots and rapid recovery; — = no visible damage.

Experiment 3

This experiment was made to observe the effect of mild, but frequent freezing on poplars. Ten young trees, 2–4 years old, of *P. eugenei*, were prepared as in Exp. 1 and frozen for 6 hr. every fortnight between 13 March and 22 May 1950. The temperatures used were as follows:

Exposure no.	Date	Temperature ($^{\circ}\text{C}.$)
1	13. iii. 50	—6
2	27. iii. 50	—3
3	11. iv. 50	—3
4	24. iv. 50	—3
5	8. v. 50	—1
6	22. v. 50	—1

Freezing damage was limited to the leaves, and was observed after the third and fourth exposures on 11 and 24 April 1950 respectively. Necrotic spots were observed on the affected plants, which recovered completely during the following summer. No damage of any kind was observed on the stems or branches of the young trees.

INTERNAL DAMAGE

In the leaves

Frost injury to the leaves took the normal form of cell breakdown and lifting of the epidermis in the necrotic areas.

In the twigs

Twigs less than 1 year old, collected from plants frozen once at -3°C . on 16 May 1949 were fixed and sectioned. Twigs, 1–3 years old, were also collected from plants frozen 6 times (Exp. 3) about 2 days after the last freezing and fixed in formalin-acetic alcohol. The material was dehydrated in ethyl alcohol, embedded in celloidin, sectioned (20μ) and stained with safranin and fast green.

In some of the young twigs (frozen once at -3°C .) the bast fibres were detached from the adjacent cells. The tips of the outer bud leaves were disorganized and discoloured as previously described. Below the buds, regions of disorganized parenchyma were found at a point corresponding with the passage of the leaf trace into the cortex. In the older twigs (frozen 6 times), a few small rents were observed in the outer layers of the medulla, but the wood was undamaged. Occasionally an indication of abnormal wood, as described by Day & Peace (1934*b*) in other trees, was observed. This wood showed newly formed vessels which were either smaller or larger than normal. A deeply stained substance was sometimes observed in the medullary rays and some of the vessels. Some of the fibre bundles in the bark were detached from the surrounding tissues, and some of the cortex parenchyma showed slight discoloration, especially in the few layers near the periderm, which itself was not affected.

INOCULATION EXPERIMENTS

Experiment 4

The plants used were those frozen in Exp. 1 to which nine controls were added, making a total of thirty-six. The natural bacterial slime (Sabet & Dowson, 1952; Sabet, 1953) exuding from diseased twigs, was distributed on dead and living branches of the plants with a camel-hair brush. The plants were then kept for a week in a saturated atmosphere at 18°C . No infections resulted.

Experiment 5

Four lots, each of ten plants of *P. eugenei*, were prepared as follows: in the first lot, the plants frozen 6 times (see Exp. 3) were inoculated with fresh natural slime; five were inoculated on 14 May 1950 and five on 26 May 1950. These plants were placed after each freezing in a frost-proof greenhouse. The plants of the second lot (not frozen) were also kept in the frost-proof greenhouse and were inoculated on 17 May 1950 with fresh natural slime. In the third lot, plants naturally affected with the bacterial disease were frozen 6 times, in exactly the same manner as in the first lot, and placed in the frost-proof greenhouse after each freezing. The fourth

lot consisted of ten unfrozen plants naturally affected with the bacterial disease to approximately the same degree as in the previous lot.

Table 2 shows that no infection took place in the five plants of the first lot inoculated between the fifth and sixth freezings, but three out of the five plants inoculated after the last freezing were infected. Of the second lot (plants inoculated and kept in a frost-proof greenhouse), seven plants out of ten were infected. In the third lot (freezing plants already infected), six plants developed new cracks in the spring and died in the summer, while four plants developed new cracks and were affected with die-back. In the fourth lot two plants developed new cracks in the spring and died in the summer while some of the branches of the remaining eight plants were affected with die-back.

TABLE 2. *The effects of freezing on infection and progress of the disease in Populus eugeni*

	Treatment	Observations
1st lot of 10 healthy plants	Frozen 6 times:	
	(a) 5 plants inoculated between 5th and 6th freezing	(a) No infection
	(b) 5 plants inoculated after 6th freezing	(b) 3 infections
2nd lot of 10 healthy plants	Inoculated	7 infected
3rd lot of 10 diseased plants	Frozen 6 times	6 developed new cracks and died, 4 developed cracks and die-back
4th lot of 10 diseased plants	—	2 developed new cracks and died. 8 developed new cracks and die-back

DISCUSSION

In the foregoing experiments, it has been shown that artificial freezing produces a serious necrosis of leaves. The branches, on the other hand, were either killed outright or else showed only slight internal damage. No cracks or cankers were formed on living branches. There are two features of the internal damage which may be connected with the bacterial die-back and canker: (i) the slight formation of abnormal wood, and (ii) the reddish discoloration in the wood. Day (1948) has called attention to the production of abnormal wood and the reddish discoloration in both the bacterial disease of poplar and frost internal damage. He concluded that natural frost is the primary pathogenic factor on the ground that these symptoms are characteristics of frost damage. In fact, both these morbid features may be developed, not only by frost, but also by other unfavourable environmental conditions, such as mechanical injury or disease and insects, and should not be considered alone as diagnostic characters.

The present investigation suggests that the relation between freezing and the bacterial canker may be limited to later stages of the disease. That freezing did not cause any opening for infection (Exp. 4) and may inhibit infection in an early stage,

and that infection takes place in plants protected from frost (Exp. 5), suggest that frost is not essential for the establishment of infection. It is believed, on the other hand, that frost may increase the aggressiveness of the disease in its post-infection stages as do other environmental factors such as rain and high relative humidity. This view is supported by the last experiment, which showed that plants already infected with the bacterial disease suffer more by freezing.

The writer wishes to express his sincere indebtedness to Dr W. J. Dowson under whose supervision the work was carried out, and would like to thank Mr T. R. Peace of the Forestry Commission for his interest and useful suggestions.

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DEPOSITION OF AIR-BORNE *LYCOPODIUM* SPORES ON PLANE SURFACES

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(With 12 Text-figures)

The deposition of *Lycopodium* spores on sticky surface traps, including vertical and horizontal microscope slides and Petri dishes as used in routine aerobiological survey, was studied in a small wind tunnel at wind speeds from approximately 0.5–9.5 m./sec.

Deposition results from several processes acting singly or in combination. The pattern of deposit on a microscope slide orientated at angles varying from 0 to 90° to the wind, with gravity either positive, neutral or negative, indicates that, except on a surface parallel to the wind at the lowest wind speed, sedimentation under gravity plays a minor part in deposition on plane surfaces. As the wind speed is increased the deposit is decreased because of 'edge shadow'. At the highest wind speed the deposit is as large on the lower as on the upper surface of the horizontal slide, which suggests deposition by turbulence. Deposition by impaction against a vertical strip increases with wind speed, and efficiencies observed are lower than for cylinders of the same diameter. The deposit on slides inclined at angles of 45° or less to the wind direction is increased by impaction of an 'edge drift'. The interaction of these various deposition processes on mean deposition at different angles and wind speeds gives a series of curves with a maximum at 90° at 9.5 m./sec. and with minima at 0, 90 and 180° at wind speeds lower than 9.5 m./sec. At these lower wind speeds there are two maxima in the range 20–70° and 135–150° respectively.

Deposits on Petri dishes show rim effects differing at different wind speeds. These can be eliminated by sinking the dish below a flat surface.

Blow-off from non-sticky surfaces is least at about 45°, and greatest at 0°.

Spore concentration in air is difficult to estimate from the deposits on plane-surface traps, because horizontal traps under-record at medium wind speeds, and because vertical traps are very sensitive to changes in wind speed. Power-operated suction traps are to be preferred when data on spore concentrations are required.

INTRODUCTION

Experiments on the deposition of air-borne *Lycopodium* spores on vertical sticky cylinders from clouds of known concentration in a small wind tunnel have already been reported (Gregory, 1951). This paper summarizes the results of over 1000 wind tunnel experiments on the deposition of *Lycopodium* spores on plane surfaces, with the following variables: (1) wind speed; (2) size, shape, and orientation of surface; and (3) effect of adhesive coating. The ultimate object of the investigation is to understand the mechanism by which fungus spores and protectant dusts are deposited on plant surfaces, but the present phase is concerned with deposition on artificial surfaces. The opportunity was taken to calibrate the surface traps used in

routine aerobiological survey, such as vertical and horizontal glass microscope slides and Petri dishes.

Current interpretation assumes that particle deposition on a plane horizontal surface is due almost entirely to gravity (Cocke, 1937, 1938; Durham, 1944). The investigation shows, however, that deposition of a spore on a plane surface may result from the operation of one or more mechanisms.

In its general form the theory of gravity deposition assumes that the air flowing past the surface contains a large population of particles distributed at random. The particles fall at their terminal velocity, v_s cm./sec., and the wind blows horizontally at v_o cm./sec. A plane surface of area 1 cm.² faces the wind, making an angle θ with the horizontal planes as in Fig. 1. Then only particles contained in the rectangular

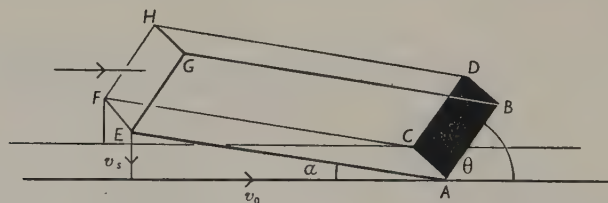


Fig. 1. Diagram illustrating usual gravity theory of particle deposition. $ABCD$ = trap surface; v_o = wind velocity; v_s = terminal velocity of particle; $ABCDEFGH$ = rectangular skew prism containing particles whose trajectories would bring them to rest on trap surface; θ = presentation angle.

skew prism A, B, C, D, E, F, G, H , have trajectories in the free air which would carry them to the surface during time t sec. The volume of this prism is given by

$$V = t(v_o \sin \theta + v_s \cos \theta) \text{ c.c.}$$

If $\theta = 90^\circ$, then $v_s \cos \theta = 0$, and hence the number of particles with trajectories cutting a vertical surface should be independent of the terminal velocity of the particles but will depend on the wind run. (Deposition will in practice be reduced below this value because the air stream is deflected by the surface itself, and efficiency of collection has been shown to depend on v_o (Langmuir & Blodgett, 1949; Gregory, 1951).) If $\theta = 0^\circ$, then $v_o \sin \theta = 0$, and deposition under gravity should depend on the terminal velocity, v_s , so that for a horizontal trap the volume of air sampled should be independent of wind speed and should depend only on the terminal velocity of the particles. From a cloud of uniform concentration the greatest number of trajectories should pass through a surface inclined at an angle θ when $\tan \theta = v_o/v_s$ ($\theta = 45^\circ$ when $v_s = v_o$).

The discussion which follows depends upon the measurement of two quantities: (1) the 'area dose' (Landahl & Hermann, 1949); and (2) the 'trap dose'. The 'area dose' is the number of particles passing through an area of 1 cm.² in a plane at right angles to the wind. The 'trap dose' is the number of particles deposited on

1 cm.² of trap surface. (The 'area dose' must be distinguished from the 'area dosage' of insecticide workers, which is the volume of spray applied per acre.)

It will be convenient to measure collection efficiency (E) of plane surfaces by expressing the observed 'trap dose' as a percentage of the observed 'area dose' (p. 654).

If the time mean density of spore cloud = C_t spores/m.³, it will be apparent that 'area dose', A.D. = $C_t v_o$ on the gravity deposition theory, and that 'trap dose', T.D. = $C_t v_s$. From which, if the deposition is by gravity as assumed, $E\%$ A.D. expected = $(100/v_o)v_s$. Comparison of observed results with the expected value will give a convenient test of the validity of the theory.

Cocke (1938) first attempted to verify the relation experimentally with a simple apparatus which sucked a known volume of outdoor air over sticky plates, and he claimed good agreement with pollen content of the air as calculated from a horizontal slide exposed at the same time. Hawes, Small & Miller (1942) used an improved suction method and found that the mean catches on the horizontal slide were higher than estimated from the suction trap. This they attributed to the turbulence of the air-flow over the slide which is greater on windy days, a conclusion substantiated by our experiments in the wind tunnel.

Durham (1944) made a detailed study of air-borne pollen in Chicago, making simultaneous measurements with the 'gravity slide' and with two types of suction trap, the Keitt & Jones (1926) filter and the Hawes *et al.* (1942) volumetric impinger. He found good agreement between the two types of suction trap, but reported discrepancies of up to 7 times between them and the gravity slide. Later, when he noted that catch varied with wind speed, he modified the shelter for exposing the slide and claimed better agreement with the suction traps (Durham, 1946). His data, however, still indicated discrepancies of nearly 7 times. The magnitude of discrepancy varied from day to day, and one contributory factor may be that the necessity of operating the suction trap isokinetically was not recognized. Further, from our wind-tunnel data with *Lycopodium* it appears that the gravity slide seriously underestimates spore concentration at moderate wind speeds. Landahl & Hermann (1949), as a result of wind-tunnel experiments on deposition of aerosols, conclude that 'particles over a wide range of sizes settle as though in quiet air even though the air is flowing turbulently'. Durham (1946), however, found a deposit on the underside of the slide in some conditions, and realized that turbulence must play a part in deposition. Gilbert (1950) found that volumetric sampling with a bubbling apparatus indicated up to 12 times the concentration of pollen estimated by the gravity slide.

METHODS

The experiments were done in the small wind tunnel described previously (Gregory, 1951). The test particles were spores of *Lycopodium*, about 28μ diameter, numbering $9.39 \times 10^7/g$. Mr W. L. Dennis, Chemical Defence Experimental Establishment,

Porton, kindly determined their terminal velocity in air as 1.42 cm./sec. at approximately N.T.P., using the direct method with a sample of our batch of *Lycopodium* spores. This value is lower than that of 1.76 cm./sec. previously adopted.

About 50–80 mg. of spores, weighed to within 0.2 mg., were used in each experiment, and results computed on the common basis of 10^6 spores liberated from the input tube. The input mechanism used in the earlier experiments was abandoned in favour of an input using the 'boiling bed' principle. In the form of input finally adopted a weighed quantity of spores was placed on a filter-paper diaphragm which was fitted across a small thistle funnel below the floor of the tunnel. A stream of air under constant pressure was passed through the filter-paper, producing a rising cloud of spores that passed up into a bulb, from which aggregates fell back while single spores were accelerated up a narrow straight glass tube, opening on the axis of the tunnel. The original pair of grids used to make the flow turbulent was replaced by a single symmetrical grid which gave equal diffusion in a vertical and horizontal plane. With both turbulent and partially streamlined winds down to 0.5 m./sec., this arrangement gave a wider and more reproducible spore cloud on the axis of the tunnel than the original input device.

Deposits were measured by direct counting of sample areas under a microscope at a magnification of from 120 to 200 diameters. This method is preferred to indirect methods of estimation which introduce assumptions of doubtful accuracy. Differences in deposit of the order of 10,000 times can be measured directly by altering the area sampled. Deposits on the traps were sparse, at the densest not more than 10% of the surface was occupied by spores, and any effect of the deposit itself modifying the properties of the trap could therefore be neglected.

The area dose (A.D.) per million spores liberated was measured at the trapping position, 140 cm. from the input position on the axis of the tunnel, by use of the Cascade Impactor, operated isokinetically. With the new input and grid, observed 'area dose' values for turbulent conditions were approximately 6500 per million *Lycopodium* spores liberated, at wind speeds from 1.1 to 9.5 m./sec.

Unless otherwise stated, the traps were made sticky by coating with glycerine jelly.

Distances in the tunnel are measured from the input point (o) on the axis of the tunnel. The usual convention for axes is followed, with the x axis downwind, positive from the origin, the y -axis horizontal across the tunnel, and the z -axis vertical (gravity acts in the $-z$ direction).

MECHANISM OF DEPOSITION ON A GLASS SLIDE

Preliminary experiments with various types of horizontal surface trap showed wide divergences between deposits observed and those expected on the gravity theory. Other factors, such as turbulence, wind speed and edge effects, evidently play a part in controlling deposition. A detailed study was therefore made of how various factors affect spore deposition on a standard 3 in. \times 1 in. glass microscope slide

($7.6 \times 2.5 \times 0.13$ cm.). The slide was held by clips at the two ends to avoid disturbing the air-flow, and placed with its long axis at right angles to the wind. Its surface was orientated at different angles to the wind in different experiments, the convention adopted being: presentation angle 0° = parallel with wind, 45° when the leading edge was lower than the trailing edge (angle θ in Fig. 1) and 90° = at right angles to wind. Presentation angle so defined differs from the aeronautical 'angle of incidence' in which the leading edge is higher than the trailing edge at 45° .

The effect of gravity was studied in two sets of experiments. In one set, with the long axis of the slide vertical (parallel with the z -axis) and the surface intersecting the xz -plane at various angles, the effect of gravity on deposition must be neutral. In the other set, with the long axis of the slide horizontal (parallel with the y axis) and the surface intersecting the xy plane at various angles, the effect of gravity must be positive at angles from 0° up to less than 90° , neutral at 90° , and negative at angles greater than 90° up to 180° . (Angles greater than 180° represent the back of the slide.)

The deposit was counted by scanning successive half centimetre zones across the 2.5 cm. width of the slide from the leading edge (zone A) to trailing edge (zone E). In Fig. 2 the efficiency of deposition on each of the five zones across the slide is shown on a logarithmic scale: for six wind speeds from 0.5 to 9.5 m./sec.; at seven angles from 0 to 90° ; and for gravity positive, neutral and negative. Most of the observations are based on a single reading as replication proved impracticable. Values below $E=0.1\%$ are unreliable, but the observed values are given to show the trend. Values below 0.01% including zero are plotted as 0.01% as they cannot be reasonably distinguished. The efficiency of deposition expected on the gravity theory at 0° for each wind speed, taking $v_s = 1.76$ cm./sec., is indicated by dotted lines.

The curves obtained probably result from the interaction of several deposition mechanisms: sedimentation, impaction, turbulence, edge shadow and edge drift. In certain sets of conditions one or other of the mechanisms can be found acting singly, in most combinations of conditions, however, deposition is interpreted as resulting from the simultaneous action of several mechanisms.

Sedimentation under gravity

Deposition by gravity alone is seen at 0° , 0.5 m./sec., with gravity positive ($g+$), i.e. on the upper surface of a horizontal slide at the lowest wind speed tested. At the same presentation angle and wind speed, deposition is zero when the effect of gravity is neutral or negative (i.e. on the lower surface of a horizontal slide, or on either side of a vertical slide parallel with the wind). With gravity positive deposition over most of the slide approximates to the value predicted by the gravity theory, but there is evidence of edge shadow reducing the deposit near the leading edge.

At 0° , 1.1 m./sec., $g+$, deposition is still entirely due to sedimentation under gravity, but at this wind speed the bluff edge of the slide (0.13 cm. thick) evidently

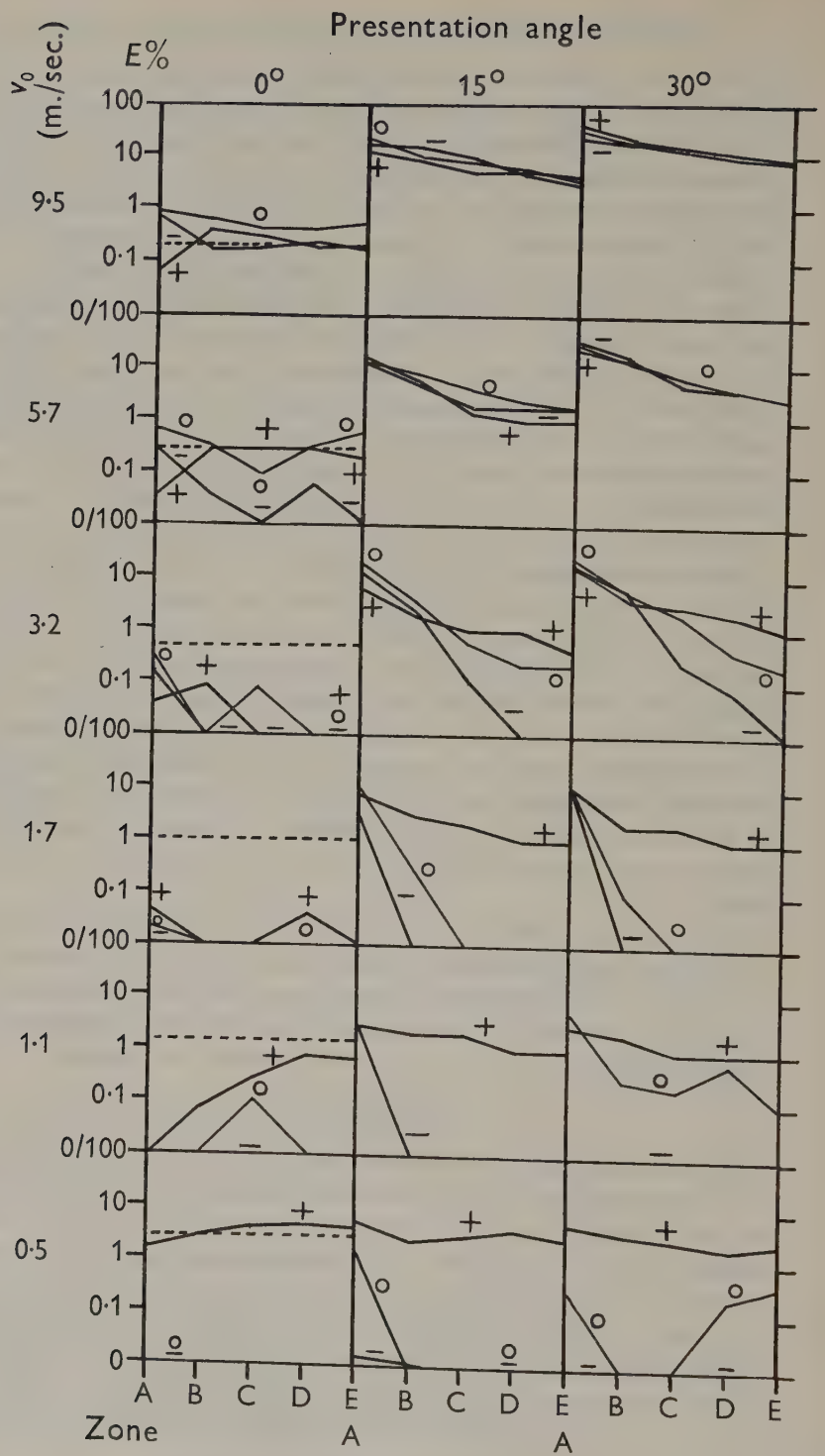
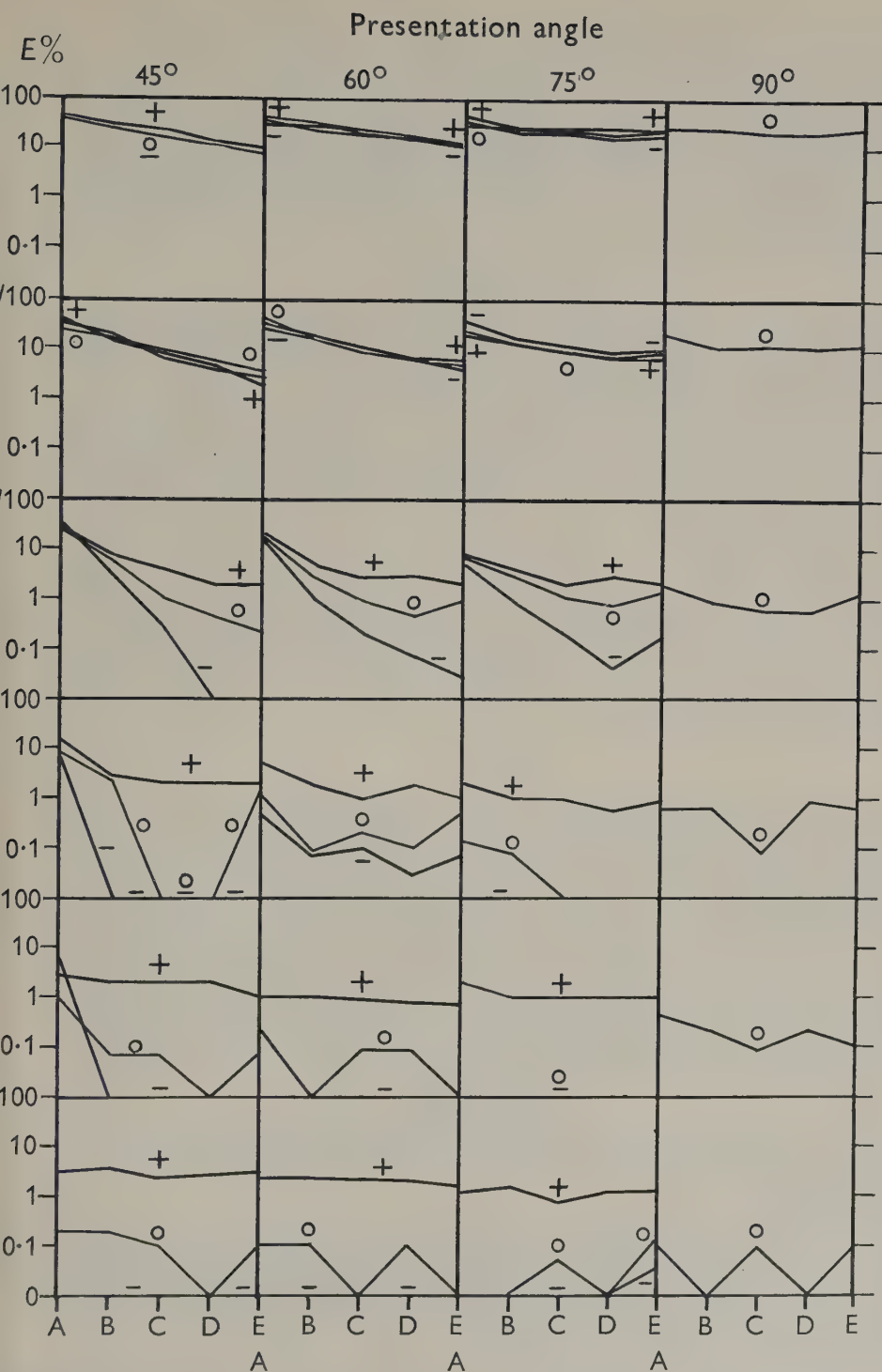


Fig. 2. Efficiency of deposition of *Lycopodium* spores on zones across glass as percentage area dose; A, B, C, D, E=successive half centimetre zones; o=gravity neutral; - = gravity negative.



at presentation angles from 0 to 90° as observed in wind tunnel experiments. E = efficiency
 e from leading edge (A) to trailing edge (E); v_o = wind velocity; + = gravity positive;

produces an upward current of air sufficient to deflect most of the spores from their course (edge shadow effect) so that deposition on the leading edge is zero, and most of the spores are even thrown clear of the trailing edge.

At 0° , with speed of 1.7 m./sec. and over, the whole surface is edge shadowed, and gravity deposition of dry *Lycopodium* on a horizontal slide is far below expectation. At higher wind speeds deposition increased again as turbulence develops.

Turbulent deposition

Deposition by turbulence generated by the slide itself is seen in pure form at 0° , 9.5 m./sec. Under these conditions deposition is approximately the same whether the action of gravity is positive, neutral or negative.

At 0° , 5.7 m./sec., although deposition is turbulent, gravity appears to interact with the process in some way at present obscure. It is supposed that when the turbulence is generated by the edge and surface of the slide many of the particles are thrown in the direction of the surface fast enough to cross the laminar layer even against the action of gravity.

With gravity neutral at 0° , 9.5, and 0° , 5.7 m./sec., deposition is higher at the leading and trailing edges than in the middle, possibly because of stationary eddies forming edge drifts. The leading edge shows an anomaly, having a deposit with gravity negative of 8–10 times the deposit of gravity positive. The anomaly occurs also at 0° , 3.2 m./sec. With gravity negative at 5.7 and 3.2 m./sec., deposition behind the leading edge is negligible.

Impaction

Deposition by impaction should be zero at 0° , but it would be expected to occur to some extent at all other presentation angles as the surface subtends the oncoming air stream. With the slide vertical (90°), the gravity effect should be neutral, and at low wind speeds of 0.5 to 1.1 m./sec. the slide would not be expected to cause turbulence. Deposition under these conditions should be due to impaction only. As wind speed is increased (90° , 1.7–9.5 m./sec.) deposition increases over the whole surface and is more uniformly distributed, but even at the highest speed tested the deposit at the margin exceeds that at the middle. In this respect impaction on plane surfaces contrasts strikingly with that on cylinders where the centre of the trace is always denser than the edges (Gregory, 1951).

Edge drift

The effect of the bluff edge of the slide in 'shading' the leading edge has been referred to above.

Behind the edge shadow a region of greater deposition due to edge drift might be expected. At 0° the edge drift evidently falls behind the trailing edge, but when the slide is inclined at 15° or 30° to the wind, the edge drift will be impacted on the slide. This is shown by the deposit on the leading edge which greatly exceeds the expected value over the range 15 – 60° , 1.7–9.5 m./sec.

Mixed effects

Over most of the range of zones, presentation angles and wind speeds, the deposit presumably results from a mixture of two or more mechanisms whose relative importance can be roughly assessed from the empirical data shown in Fig. 2.

Orientation of spores

The *Lycopodium* spores show different orientations in different parts of the deposit. Gregory (1951) stated that in the stagnation line upwind on a vertical cylinder the spores lie with the rounded distal surface uppermost, and that spores settling in air under gravity come to rest in the same position. Further observation shows that this was incorrect and that in the stagnation zone or its equivalent the spore lies with the rounded distal surface touching the surface. Evidently the spore becomes orientated with the point trailing as it moves through the air. Orientation with the point upwind is therefore characteristic of *Lycopodium* in the stagnation zone.

Microscopic observation of deposits shows that, as expected, when the glass slide is horizontal the stagnation zone is on the edge, and when the slide is vertical it is in the middle of the slide. At intermediate presentation angles, seen most clearly at angles near 90° , the stagnation zone shifts, at 115° occupying zone B, and reaching zone A at about 120° at the higher wind speeds. At lower wind speeds orientation is less definite.

MEAN DEPOSIT ON INCLINED SLIDES

The efficiencies of various kinds of spore trap under different conditions have been measured and interpreted in terms of the deposition mechanisms described in the previous section. In practice, when scanning a slide or Petri dish trap the surface is not divided into zones and the interest lies in obtaining a mean value for the whole sample area.

Mean deposits on a microscope slide with its long axis parallel to the y -axis, orientated at various presentation angles to the xy -plane (i.e. $g +$ or $-$) can be obtained from data in Fig. 2 (previous section). Each point on Fig. 3 represents data obtained from one to eight experiments.

The main effect observed in tests with inclined slides in turbulent wind is the consistent increase in efficiency with increasing wind speed at all angles, except at 0° .

The effect of inclining the slide depends greatly on wind speed. With a wind of 9.5 m./sec., efficiency is at a maximum when the slide is vertical (90°). As the angle is changed the deposit decreases more or less symmetrically on each side of the maximum until similar low values are reached with the surface horizontal (0°) or inverted (180°)—positions already discussed as the upper and lower surface on

which deposition at this wind speed was attributed entirely to turbulence. At lower wind speeds the curve shows two maxima, shifting from about 75° and 105° at 5.75 m./sec. to about 25° and 150° at 1.1 m./sec. Fig. 2 shows that these two maxima are due to a heavy deposit on the leading edge (edge drift).

At 5.75 m./sec., deposits at the two maxima are approximately equal, whereas at lower wind speeds the deposit at $25\text{--}40^\circ$ is larger than that at $135\text{--}150^\circ$, presumably because at low wind speeds gravity has a relatively greater effect in increasing the deposit on upward-facing surfaces, and decreasing it on downward-facing surfaces,

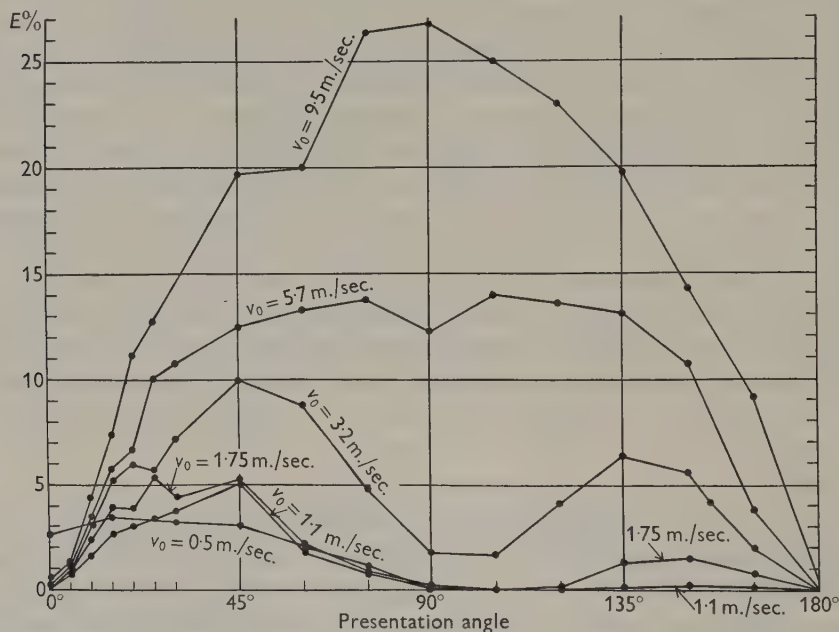


Fig. 3. Mean efficiency of deposition of *Lycopodium* spores on glass microscope slide (all zones) at presentation angles $0\text{--}180^\circ$. E = efficiency as percentage area dose; v_0 = wind velocity.

as would be expected from the values for 30° , 3.2 m./sec. for $g+$, $g0$ and $g-$ in Fig. 2.

Deposition on the back of the microscope slide was estimated approximately at selected presentation angles between 180 and 360° and, as shown in Table 1, efficiency was usually less than 1% .

Variability between replicate runs was greater with certain presentation angles than with others. For instance, values of E obtained in eight consecutive runs at 90° , 9.5 m./sec. were 24.5 , 24.1 , 26.6 , 25.6 , 34.0 , 22.9 , 26.3 , 27.8% (mean $E = 26.7\%$) respectively. At lower wind speeds and smaller presentation angles the variability increased, for example three consecutive runs at 0° , 3.2 m./sec. gave 0 , 0.07 , 0.026%

respectively (mean = 0.05 %). At the nominal angle of 0° slight errors in orientation may introduce large differences in flow pattern as noted by Simmons & Dewey (1930).

The values shown in Fig. 3 were obtained with highly turbulent wind. Partially streamlining the flow by removing the turbulence grid from the tunnel had little effect on the deposit except at presentation angles between 0 and 10°, and wind speeds below 5 m./sec. Efficiency of deposition is then reduced, being least with the horizontal slide (0°).

TABLE 1. *Efficiency of deposition of Lycopodium spores on back of microscope slide in turbulent wind tunnel ($E = \% \text{ A.D.}$)*

Wind speed (v_0 m./s.)	Presentation angle				
	210°	240°	270°	300°	330°
9.5	0.1	0.05	0.1	0.05	0.05
5.7	0.1	0.05	0.2	0.05	0.05
3.2	0.05	0.1	0.2	0.2	0.1
1.7	0.02	0.1	0.1	0.3	0.3
1.1	0.05	0.001	0.1	0.9	1.0
0.5	0.0	0.0	0.01	1.1	1.5

Deposit on strip 0.5 cm. wide

The efficiency of catch at corresponding wind speeds and presentation angles was increased by reducing the width of the slide from the customary 2.5 to 0.5 cm., and the increase was greatest at the lowest wind speeds (Fig. 4). The narrow trap was most efficient at 5.5 m./sec. and 90°. The fall in efficiency at 9.5 m./sec. was comparable with the anomalous reduction in efficiency with very narrow cylinders at higher wind speeds (Gregory, 1951). The effect of gravity did not become noticeable until the wind speed was reduced to 1.1 m./sec.

EFFICIENCY OF HORIZONTAL TRAPS

To measure the efficiency of deposition of *Lycopodium* spores on horizontal traps in routine use, and to explore possible improvements, the following surfaces were tested: (1) thin gravity slide; (2) thick gravity slide; (3) sharp plate; (4) rectangular microscope cover-glass; (5) double-edge safety razor blade; (6) circular microscope cover-glass; (7) stainless steel cutting disks (3 patterns); (8) Petri dish.

The deposit of *Lycopodium* spores expected at various wind speeds according to the 'gravity' hypothesis is shown on a double logarithmic scale by the straight lines *a* and *b* (Figs. 5, 6), calculated as $E \% \text{ A.D.} = 100 v_s/v_0$, and assuming terminal velocities of 1.76 and 1.42 cm./sec. respectively. The observed efficiencies (mean for whole surface) of the traps are also shown on Figs. 5 and 6, and the distribution in zones on certain traps is shown in detail in Fig. 7.

Thin gravity slide

A glass microscope slide, $7.6 \times 2.5 \times 0.13$ cm., was placed horizontally with its long axis: (a) parallel to the y -axis of the tunnel (data from previous section); and (b) parallel to the x -axis.

This approximates to the arrangement used by Hyde (1950) for pollen trapping. Deposits on various parts of the surface with the wind flowing, unobstructed by supports or clips, either across or along the length of the slide are shown in Fig. 7.

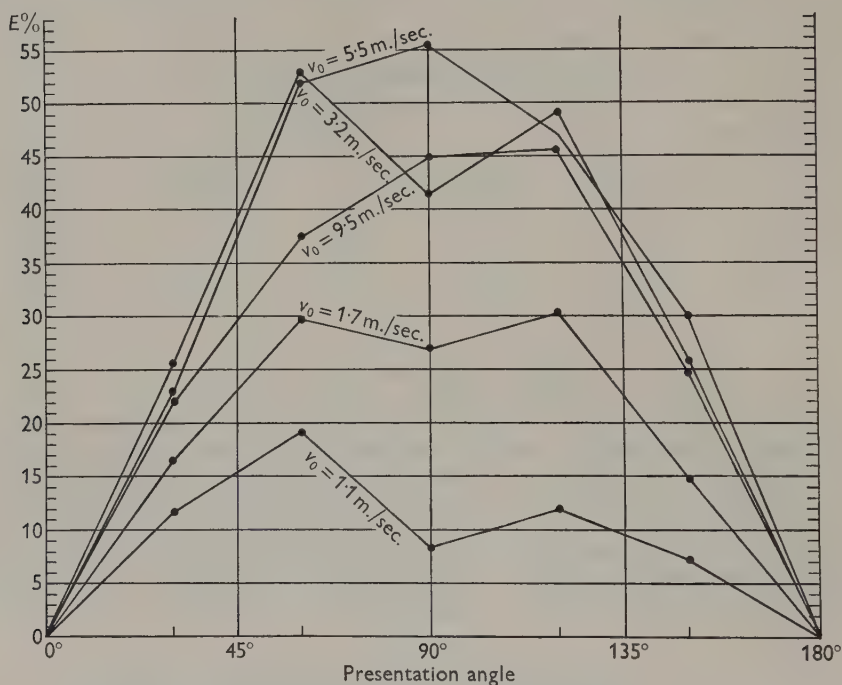


Fig. 4. Mean efficiency of deposition of *Lycopodium* spores on glass strip 0.5 cm. wide, at presentation angles 0–180°. (Compare Fig. 2.)

At the lower wind speeds, because of edge effects, the deposit is smaller than the expected value at the leading edge, but larger at the trailing edge. As the wind speed is increased to 3.0 m./sec. the regions of small and large deposit move towards the trailing edge, and the deposit (edge shadow) is negligible in the usual sampling area when the wind is blowing across the width of the slide. Turbulence, however, again increases the deposit on the sampling area at higher wind speeds, and at 9.5 m./sec. it is considerably above the expected value. Because of edge shadow observed deposits on the sampling area decline steadily below expectation as wind speed rises above 1 m./sec., falling to about one-tenth of the expected value at 3.0 m./sec. when the wind blows across the short axis of the slide, and one-quarter

to one-third of the expected value with the wind along the length of the slide at 1.7–5.5 m./sec. A comparable series of tests with the tunnel streamlined showed similar effects with deposit decreasing to approximately one-thirtieth of the expected value at 3.2 m./sec.

Thick gravity slide

A bluff-edged Perspex plate, $7.6 \times 2.5 \times 0.6$ cm., was tested with the wind flowing either along or across the long axis (Fig. 7).

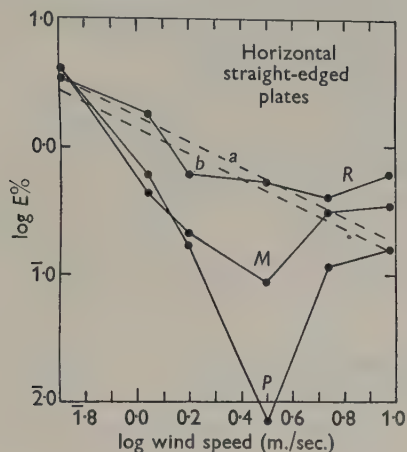


Fig. 5.

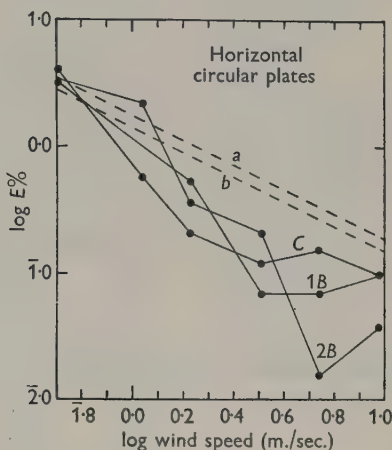


Fig. 6.

Fig. 5. Mean trapping efficiency for *Lycopodium* on horizontal rectangular plates (on double logarithmic scale). *a*, *b*, = efficiency expected on gravity theory (for $v_s = 1.76$ and 1.4 cm./sec. respectively); *R* = observed efficiency of double-edged safety razor blade; *M* = observed efficiency across thin microscope slide; *P* = observed efficiency on plate 0.6 cm. thick.

Fig. 6. Mean trapping efficiency for *Lycopodium* on horizontal disks (on double logarithmic scale). *a*, *b*, as in Fig. 5; *C* = observed efficiency of glass cover-slip, 1.9 cm. diameter, 0.016 cm. thick; 1*B* = steel disk, 5.6 cm. diameter, 0.12 cm. thick, bevelled on lower surface; 2*B* = double-bevelled steel cutting wheel, 7.6 cm. diameter, 0.14 cm. thick.

This trap approximates to the improved pollen trap used by Durham (1946), the spore trap of Hyre (1950) and others, in which a microscope slide is placed on a block making a total thickness of about $\frac{1}{4}$ in. With this trap the thickness of the effective edge of the slide is increased and edge shadow at medium wind speeds becomes more pronounced. As shown in Fig. 7, at 0.5 m./sec. there is an edge shadow 2–3 cm. wide followed by an edge drift extending up to the trailing edge with its maximum deposit near the centre of the plate. At 1.1 m./sec. the whole surface, except the rear 2 cm., is in the shadow of the leading edge, while at 1.7 m./sec. the whole surface is shadowed, and therefore under-records. At 3.2 and 5.5 m./sec. nothing is deposited on any zone. At 9.5 m./sec. there is some deposition presumably

because of turbulent drift. As with the thinner horizontal slide, the deposit at 9.5 m./sec. is the same on the upper and lower surfaces, so that deposition at this speed cannot be due to gravity. The thicker edge must disturb air flow more than the microscope slide exposed without a holder, and this presumably accounts for turbulence beginning to affect deposition at the higher wind speeds.

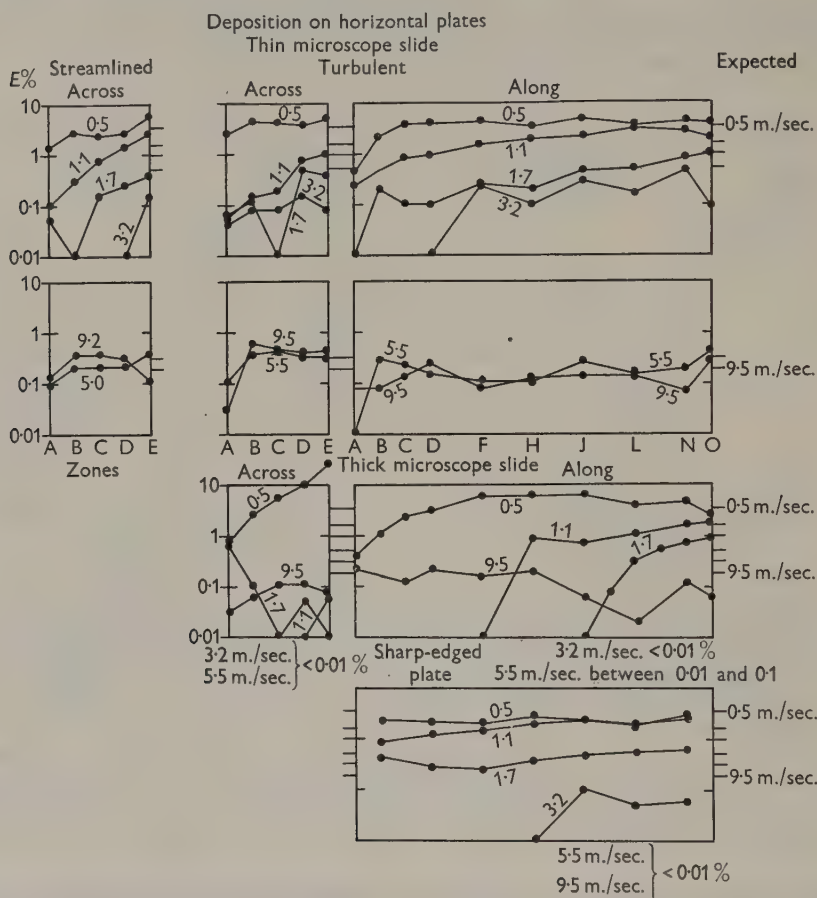


Fig. 7. Observed efficiency of deposition of *Lycopodium* on $\frac{1}{2}$ cm. zones across horizontal plates from leading edge (A); at wind speeds from 0.5 to 9.5 m./sec.; in turbulent wind tunnel. (Also streamlined wind tunnel across thin microscope slide.)

Sharp-edged plate

A 'duralumin' plate, $10 \times 7.5 \times 0.6$ cm., with one of the longer sides double-bevelled to form a 45° edge was used facing the wind. The plate was ruled in centimetre squares for convenience in recording the deposit in different zones.

The sharp-edged plate approximates to the trap used by Landahl & Hermann (1949) in wind-tunnel studies on deposition of mixed spray droplets. As shown in

Fig. 7 the horizontal surface behind the double-bevelled edge at 0.5 m./sec. receives a very even deposit over its whole surface, but at 1.1 m./sec. an edge shadow develops, and spreads across the surface as the wind speed increases, till by 5.7 m./sec. the edge drift is evidently more than 6 cm. behind the leading edge, and deposit on the slide is negligible. With this edge there is no recovery due to turbulence, and the thick sharp-edged trap underestimates by a factor of 200 times at the higher wind speeds tested. However, turbulent deposition could readily be induced in the wake of a narrow cylinder touching the surface of the plate, or placed upwind of the leading edge.

Rectangular glass plate (5.1 × 2.2 × 0.016 cm.)

A large microscopical cover-glass was used for this test, supported at each end in a horizontal position with the wind flowing across the 2.2 cm. width. Observed deposits indicate that edge-shadowing and turbulence effects occurred even with an edge as thin as 0.016 cm., but the deposits approached more nearly to the expected values than with thicker traps; they were slightly high at 0.5 and 9.5 m./sec. and only slightly low at intermediate wind speeds.

Double-edge safety razor blade

Tested with the edge to the wind, a wafer-blade gave fairly uniform deposits which were close to predicted values except at the highest wind speeds where the deposit was 3 times that expected (Fig. 5).

1.9 cm. diameter circular glass disk

A no. 1 microscope cover-glass, 0.016 cm. thick, fixed centrally on the top of a wire with 'Plasticene', had low deposits at all wind speeds above 0.5 m./sec. (Fig. 6).

Stainless steel disks

Thin sharp-edged disks of three patterns were tested: (1) 7.6 cm. diameter, 0.14 cm. thick, double-bevelled; (2) 6.3 cm. diameter, 0.12 cm. thick, flat on top, and sharply bevelled below; and (3) 5.6 cm. diameter, 0.12 cm. thick, also flat on top and sharply bevelled below.

Deposits on all were of similar order, and the spores were fairly uniformly distributed over the surface. Efficiency agreed closely with expectation at the two lowest wind speeds, but was considerably below expectation with winds above 1.7 m./sec., except at 9.4 m./sec. where efficiency again approached the expected value (Fig. 6).

Open Petri dish (diameter 9.4 cm. external, 9.0 cm. internal, 1 cm. deep)

The Petri dish, extensively used in aerobiological mould survey, was tested horizontally after pouring with 15 ml. of 2% water agar (tests showed that deposit and retention on this medium were similar to glycerine jelly). Mean deposition

efficiencies (% A.D.) for 1 cm. squares on the agar surface are shown in Fig. 8 based on a single run at each wind speed. At all wind speeds narrow edge drifts occurred behind the rim of the leading edge and in front of the rim of the trailing edge. Mean deposits for the whole dish are shown in Fig. 9. Efficiency at 0.5 m./sec. appears to be low, but the reason is not obvious. At 1.1 and 1.7 m./sec. efficiency is high, apparently because of the large contribution of the front and back edge drifts. At 3.2 m./sec. and above, efficiency falls substantially below expectation, apparently because the sampling surface is almost entirely shadowed by the 1 cm. rim of the dish. Some recovery takes place at 9.5 m./sec.

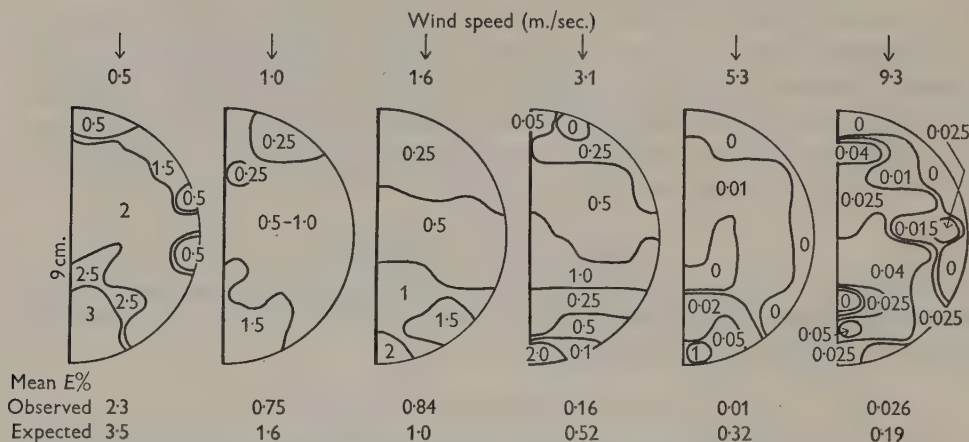


Fig. 8. Observed efficiency of deposition of *Lycopodium* on surface of horizontal Petri dish 9.0 cm. diameter. (Contours drawn from counts on $\frac{1}{2}$ cm. squares.)

When the wind tunnel is partially streamlined by removing the turbulence grid, deviations from expected efficiency become more pronounced (Fig. 9). At 0.5 m./sec. the Petri dish gives about 3 times the expected value, but it gives only one-twentieth of the expected value at 5.7 m./sec., and increases slightly at 9.5 m./sec. These deviations are probably due to edge drift at low wind speeds and edge shadow at higher wind speeds.

To eliminate the effects produced by the rim of the dish, another series of tests was done in the turbulent tunnel with the Petri dish placed at the bottom of a metal cylinder, 13 cm. deep, 11.5 cm. diameter, sunk below a flat surface consisting of a square cardboard platform cutting the xy -plane at height $z=0$. The cardboard fitted flush with the mouth of the cylinder and extended 11 cm. up and down wind. This arrangement eliminated the low efficiency previously caused by edge shadow at 3.2 and 5.5 m./sec. and deposits were slightly above expectation at all wind speeds except the lowest (Fig. 9). The high values were apparently caused by a slight drift in front of the trailing edge of the cylindrical cavity.

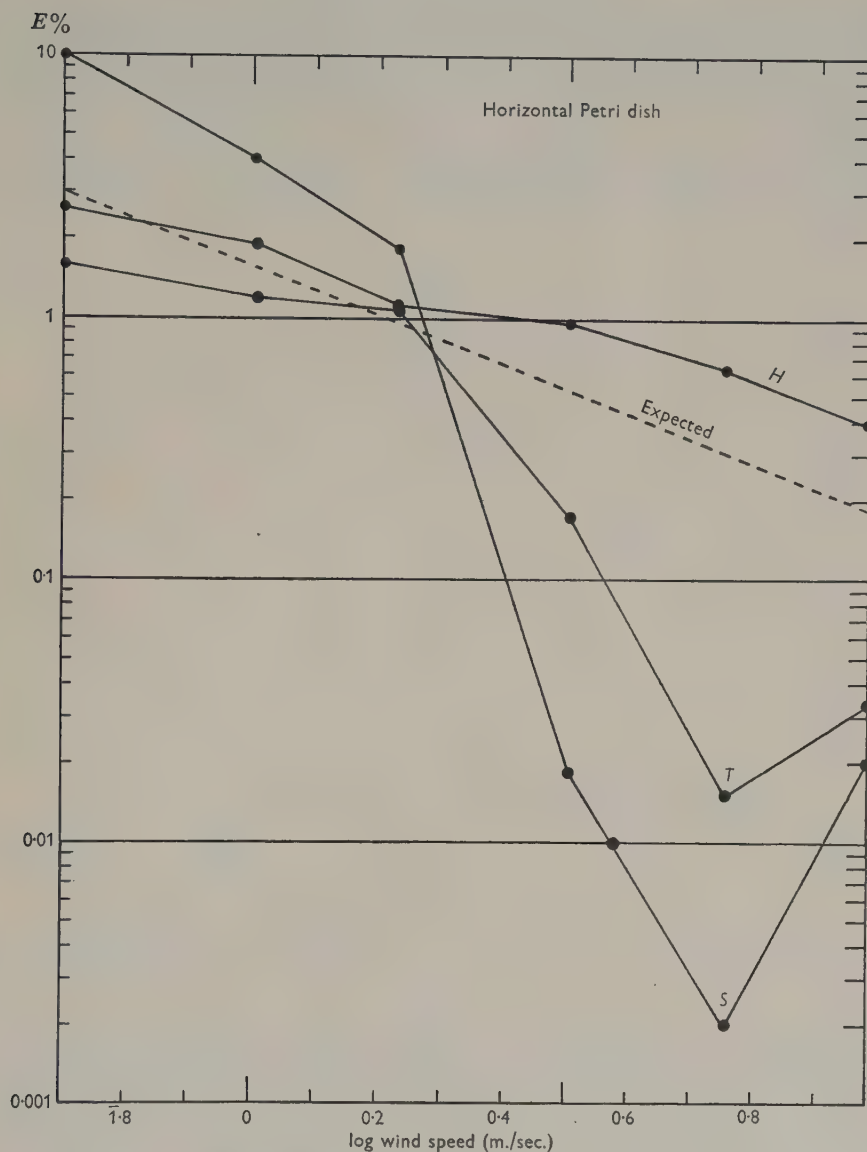


Fig. 9. Mean trapping efficiency for *Lycopodium* on horizontal Petri dish 9.0 cm. diameter (on double logarithmic scale). T =in turbulent wind; S =in streamlined wind; H =in turbulent wind with dish sunk below hole in flat plate.

DEPOSIT ON VERTICAL SURFACES

The efficiency of impaction of *Lycopodium* on a plane surface at right angles to the wind is determined by the same factors as impaction on cylinders (Gregory, 1951). Trap tested vertically facing the wind were: (1) microscope slide, $7.6 \times 2.5 \times 0.13$ cm.; (2) glass strip, $7.6 \times 0.5 \times 0.13$ cm.; (3) square glass plate, 5.9×5.9 cm.; (4) glass or celluloid disks, 9.4, 2.8, 1.3 and 0.3 cm. diameter respectively, held in position on the axis of the tunnel by a wire attached behind; and (5) Petri dish, diameter 9.4 cm. external, 9.0 cm. internal, 1 cm. deep. For a better comparison with the rimless

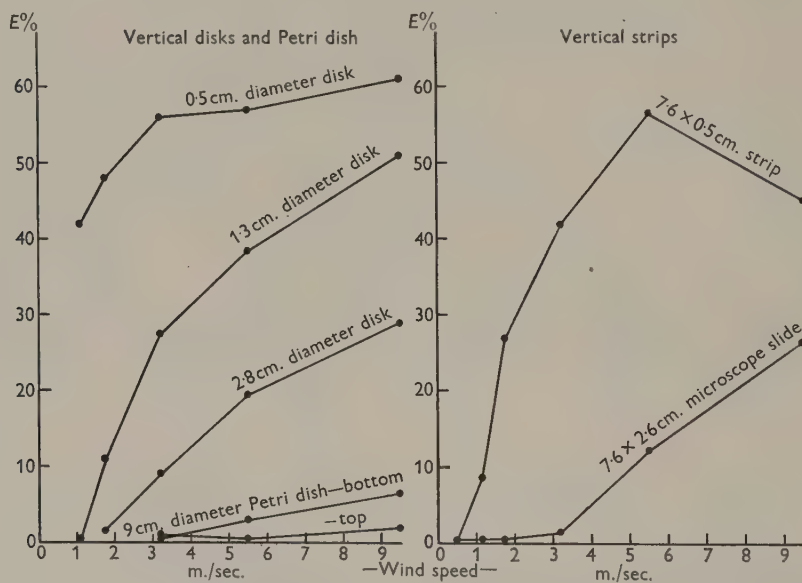


Fig. 10. Mean trapping efficiency for *Lycopodium* on vertical disks and strips as observed in turbulent wind tunnel.

disks tested earlier Petri dishes were used vertically in two positions: (a) poured with 15 ml. 2% water agar facing the wind; and (b) the bottom of the dish coated with glycerine jelly facing the wind. Efficiencies observed are shown in Fig. 10.

For a given value of k (defined as $k = v_s v_o / \frac{1}{2} dg$, where d = diameter, $g = 981$ cm./sec.²) Sell (1931) and Langmuir & Blodgett (1949) have predicted that plane surfaces would trap more efficiently than cylinders. However, tests with aerosols in a wind tunnel by Landahl & Hermann (1949) indicate that efficiencies of a plane surface were slightly lower than cylinders. In our experiments, with exceptions at the lowest wind speeds, strips received substantially less than the deposits predicted by Langmuir & Blodgett for a ribbon of infinite length with ideal fluid flow. This result agrees with the observations of Landahl & Hermann. The fact that the length of the strip was only 3 to 10 times its width would, if anything, be expected

to increase the deposit above that expected on an infinite strip. Observed values of k for the strips (Fig. 11, B) agree well with those for cylinders as predicted by Langmuir & Blodgett (1949), and observed by Gregory (1951). On the other hand, deposits observed on vertical disks agree well with the value predicted by Langmuir & Blodgett for spheres (Fig. 11, A). Deposits of *Lycopodium* spores on the back of a vertical slide were very low (Table 1).

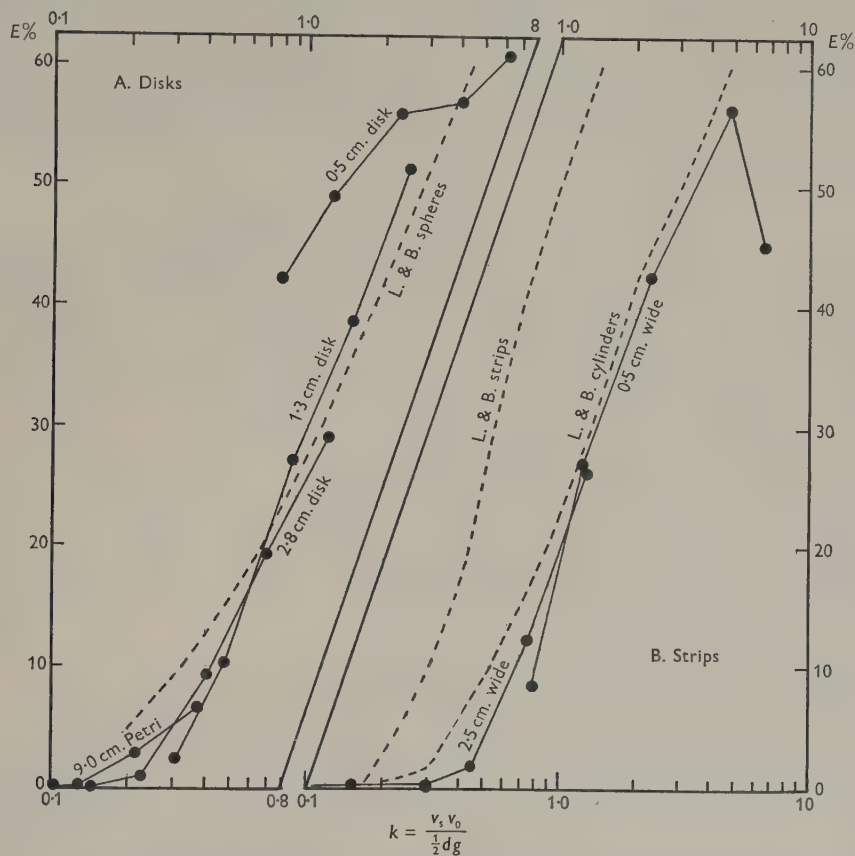


Fig. 11. Observed relation between $E\%$ and $k = v_s v_o / \frac{1}{2} dg$. (solid lines); L and B = values predicted by Langmuir & Blodgett (1949) for spheres, strips and cylinders for $\phi = 10^3$ (dotted lines).

Deposition over the surfaces of the vertical traps was not uniform. With the Petri dish at 9.4 and 5.5 m./sec., the deposit was 4 times as great in the central 5 cm. radius as in the peripheral centimetre near the rim. At and below 3.2 m./sec. the difference was reversed, with nearly 75 % more spores deposited on the 1 or 2 cm. inside the rim of the dish. The rim probably caused this reduction in peripheral deposit at the higher wind speeds, because when the dish was turned the other way

round (back to front), the deposit of the peripheral 1 cm. was 50 % higher than the central 4 cm. at 9.3 m./sec., and in this position the constant value of 0.03 % was not reached until the wind speed fell to 1.75 m./sec. This basal level of deposition is possibly the result of local turbulence rather than impaction.

Deposits on vertical traps were usually denser near the edge and lighter in the stagnation zone near the middle of the surface. This is the reverse of the cylinder effect, and is predicted by Langmuir & Blodgett (1949). *Lycopodium* spores showed different orientation on different parts of a vertical surface. On the peripheral 50 μ of the 0.5 cm. disk most spores lay with their points towards the edge, while elsewhere the spores mostly had the rounded side sticking to the surface. At the corner of the rectangular slide the spores tended to lie on their sides with their point towards the corner.

RETENTION AND BLOW-OFF FROM CLEAN SURFACES

Experiments showed that there is no loss of *Lycopodium* spores from the deposit on the surface of a slide with a sticky coating of glycerine jelly at any of the wind speeds tested. Blow-off from a non-sticky glass surface, however, depended on the wind speed and the angle of incidence to the wind. Clean microscope slides were placed in a spore cloud at 0.5 m./sec. to obtain a deposit, and were then placed successively in winds of increasing speeds. Tests were done at angles of 0°, 45° and 90°. The percentage of the original deposit retained after 1 min. at each wind speed was estimated by counting (Fig. 12). At the highest wind speed, 9.5 m./sec., slight traces of grease on the slide greatly increased retention when the slide was horizontal, and erratic results were obtained under these conditions unless the surface was carefully cleaned before use.

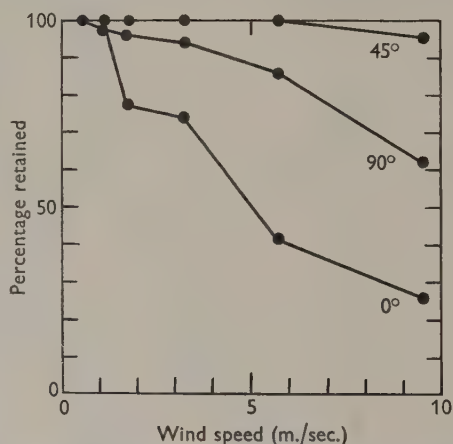


Fig. 12. Retention of *Lycopodium* on clean glass microscope slide at six wind speeds and three presentation angles observed in turbulent wind tunnel.

With the slide vertical (90°) blow-off was nearly linear with wind speed, 98 % was retained at 1.1 m./sec. and 60 % at 9.5 m./sec. Blow-off was least at 45°, and at this angle retention was 100 % up to 5.5 m./sec., and 9.5 % was retained even at 9.5 m./sec. On the other hand, blow-off was greatest with the surface horizontal (0°), when 77 % was retained at 1.7 m./sec., and only 26 % at 9.5 m./sec. Evidently these values have little application to plant surfaces.

DISCUSSION

The horizontal slide is a sedimentation trap; it has the advantage of simplicity, demands no power supply for its operation, and, in theory, it should sample various types of particles in proportion to their terminal velocity, giving a reading of time-mean concentration independent of wind speed. Tested with *Lycopodium* spores in the wind tunnel, however, edge shadow leads to underestimation by a factor of 5 to 500 times at moderate wind speeds, because the particles overshoot the sampling area by a distance which depends on the thickness of the slide, its shape, supports, and on the orientation of its long and short axes to the wind. This may be partly compensated for out of doors by turbulent deposition which occurs at high wind speeds and is not predicted by the gravity theory. Further, the defects shown in the wind tunnel, where maximum size of eddies is limited to a few centimetres, may be partly corrected in the open air, if large divergences from a horizontal wind direction are fortunate enough to lead to an increase in deposition at small angles at low and medium wind speeds (Fig. 3). The efficiency of this type of trap must therefore be tested against a suction trap of known efficiency under field conditions before the performance of the horizontal trap can be judged. Preliminary data from such a comparison carried out in 1951 and 1952 indicate that the range of variation of the horizontal slide is in practice about 10 times. Variations in the angle of incidence of the wind, and turbulent deposition during gusts probably tend to correct for under-recording observed in the wind tunnel.

It is evident that the horizontal slide can be improved by being made thin, and that any support or clips which add to its total thickness will produce edge-shadow effects, leading to under-recording at average wind speeds. A good trap should be thin, have the edge bevelled on the lower side to reduce edge shadow, and should be small enough to avoid excessive turbulent deposition on the sampling area at higher wind speeds.

Landahl & Hermann (1949) reported that the amount of aerosol 'deposited on a vertical slide does not change much if it is oriented within 30° to the wind'. Our results indicate that with *Lycopodium* spores this is true only at wind speeds of about 5 m./sec. Rotating the slide from 90 to 60° increases the efficiency 10 times at 1.1 and 0.75 m./sec.; about 5 times at 3.2 m./sec., while at 9.5 m./sec. efficiency is decreased by one-quarter.

The use of the 7.6×2.5 cm. microscope slide at angles intermediate between 0 and 90° would lead to greater trapping efficiency at wind speeds normally experienced in the open air near the ground (but not at speeds as low as $v_0 = v_s$, as the gravity theory would predict). Hyre (1950) observed that a slide exposed in the field caught more sporangia of *Pseudoperonospora cubensis* with a presentation angle of 45° than either a vertical or horizontal slide at wind speeds up to 4.8 m.p.h., a result easily explicable by the data presented in Fig. 3. It should be possible to orientate a slide at 5° to the oncoming wind, which should give an efficiency varying only between 0.6 and 1.4 % over the range 1–10 m./sec.; or at 10° when its efficiency

would vary between 1.5 and 4.5 % over the same range of wind speeds. An alternative approach would be to make the slide tilt according to the wind force over the range of angles of 10–30° as the wind speed varies from 10 to 1 m./sec., over which range it would have a nearly constant efficiency of 4 % with *Lycopodium*.

The vertical 7.6 × 2.5 cm. slide, widely used for research in plant pathology, is an impactor trap; it suffers from the defects of being relatively inefficient as an impactor at low wind speeds and highly sensitive to changes in wind speed. To avoid wrong conclusions and translate deposit into time-mean concentration, it is necessary to know the wind speed under which deposition took place, and to make the necessary correction (Johnson, 1950). This may be illustrated by considering the deposit received from a cloud containing 10,000 *Lycopodium* spores per cubic metre at two wind speeds, using data from Fig. 10. At 1.0 m./sec. impaction at 5 % efficiency would deposit about 20 spores/cm.²/hr. With an increase in the wind speed to 9.4 m./sec. the deposit would increase to about 9000 spores/cm.²/hr., a 450 times increase in deposit without change in the number of spores per cubic metre of air. A further source of error is that at low wind speeds the efficiency is low, and the catch is therefore small and has to be multiplied by a large factor, and so the error of estimation becomes great.

The sensitivity of this type of trap to wind speed explains an observation in the early history of pollen trapping. Blackley (1873), in one series of tests in his pioneer work on pollen content of the air near Manchester, caught from 15 to 20 times as much pollen on a vertical slide carried up to 1000 ft. by a kite, as on a vertical slide at 4½ ft. above the ground. He concluded that 'there seems to be a zone of atmosphere, commencing some distance above the earth, which contains a much larger number of germs and spores than is found in the lower portion of the atmosphere'. Blackley realized that the increased wind-run at 1000 ft. would lead to something like a threefold increase in deposit, but it now seems clear that the increased velocity of wind at that height would lead to an additional tenfold increase in efficiency of deposition.

The vertical slide has been extensively used for trapping from aeroplanes in flight. When trapping spores similar to *Lycopodium* at air speeds of about 176 m.p.h., $k=7$ approximately, and by extrapolation efficiency would be expected to reach 60–70 % unless the effective width of the slide facing the wind were substantially increased by its holder and supports. For a Petri dish used similarly k is approximately 3.5 and efficiency would be about 50 %. Systematic errors due to properties of the trap become less important at high wind speeds, and both slide and Petri dish are likely to give reasonably accurate measures of concentration for pollen and large spores when exposed from aircraft. The concentration of very small spores would probably still be underestimated at present ordinary commercial aircraft speeds. Used as a stationary horizontal trap near the ground the deviations of the Petri dish from expected values caused by the rim when sampling large spores can be removed by sinking the dish below a flat surface.

The data reported in this paper refer to *Lycopodium clavatum* spores, and presumably apply only to particles of approximately similar shape and speed of fall. With larger particles deviations caused both by edge effects and impaction efficiencies would be increased, and with smaller particles deviations and efficiencies should be decreased. Explanation of the efficiency values observed in quantitative terms comes within the scope of aerodynamics. For biological purposes it is at present sufficient to have a measure of deposition under various conditions, and to know the magnitude of the factors controlling it. Accurate measurements of cloud concentration by trap surfaces are difficult to attain, and there is evidence that some current methods of field sampling may be in error by a factor of 10 times at medium wind speeds.

It is clear that the deposit received on a surface from a cloud of known concentration is profoundly influenced by the size, shape and orientation of the surface itself. For some purposes in plant pathology and crop protection it is not necessary to know the cloud concentration, and tests of deposition could be made relevant if the test surface were made to imitate the actual surface studied sufficiently closely. The ultimate interest of many biologists, however, is primarily in concentration per unit volume of air, although great accuracy is not required at present. It will probably suffice at present to aim at an accuracy of one significant figure in field work, and two in the wind tunnel. For this purpose use in the field of a recording suction trap, such as that described by Hirst (1952), which allows the time of trapping to be determined with an error of ± 1 hour is of value, and where there is an electric power supply this type of trap should be used by all workers interested in aerobiological survey.

We wish to thank Dr R. Jackson for suggestions for improving spore input mechanisms, and Mr R. W. Gloyne for help with relevant literature.

CORRECTIONS TO PREVIOUS PAPERS

(1) P. H. Gregory (1951), *Ann. appl. Biol.* **38**, 357-76

p. 359. *Wind velocity profile*. The report of a region of higher velocity $1\frac{1}{2}$ in. from the wall of the wind tunnel is not substantiated by further work.

p. 365. Para. 3, line 7, for 'multiplied' read 'divided'.

p. 367. Table 5, note that coefficient *a* has not been corrected for zero.

p. 369. Orientation, see p. 659 above.

(2) P. H. Gregory (1950), *Nature, Lond.*, **166**, 487

Table 2 (heading). After: 'No. of *Lycopodium* spores deposited . . .' add per cm.².

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FUNGICIDAL ACTIVITY AND CHEMICAL CONSTITUTION

II.* COMPOUNDS RELATED TO 2:3-DICHLORO-1:4-NAPHTHAQUINONE

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(With 3 Text-figures)

2:3-Dichloro-1:4-naphthahydroquinone and several derivatives were prepared and tested in aqueous acetone solution against four fungi using a spore germination technique. The hydroquinone showed the same order of fungicidal activity as 2:3-dichloro-1:4-naphthaquinone, but was no less phytotoxic. Whilst the dibenzoyl ester and the dimethyl ether proved virtually inactive, the diacetyl ester was found to be as effective as 2:3-dichloro-1:4-naphthaquinone against *Sclerotinia laxa*, *Botrytis fabae* and *Cladosporium fulvum*, and also less phytotoxic to plum fruitlets and tomato and broad bean foliage. Reduced fungicidal activity was shown when the substance was formulated as an aqueous suspension suitable for field application.

The rates of alkaline hydrolysis of these esters have been determined, and a relationship between ease of hydrolysis and fungicidal activity is apparent. It is suggested that these compounds, whilst not inherently active, are similarly hydrolysed to 2:3-dichloro-1:4-naphthahydroquinone in the presence of a fungal esterase.

INTRODUCTION

2:3-Dichloro-1:4-naphthaquinone is one of the most active organic fungicides so far described. First prepared by Graebe (1867) it was introduced by ter Horst & Felix (1943) as a natural successor to tetrachloro-*p*-benzoquinone and proved to be an effective seed protectant. Its use on foliage and fruit, however, has been limited because of its tendency to be phytotoxic to some crops (Marsh, 1951; McNew & Burchfield, 1951, p. 363). Numerous quinone derivatives, both synthetic and naturally occurring, have been investigated with a view to exploring chemical structure-fungitoxicity relationships, and many of the results have been recently summarized by McNew & Burchfield (1951). In general, halogenation improves the fungitoxicity, decreases phytotoxicity and decreases solubility, whilst the presence of hydroxyl, methoxyl or alkyl groups *ortho* to the carbonyl group usually results in reduced fungitoxicity, often with an increase in solubility and phytotoxicity. In an investigation of the *in vitro* fungitoxic properties of some simple quinones, Read & Smith (1951) found that the introduction of chloroimide groups into the *para* positions of 1:4-benzoquinone and 2:6-dichloro-1:4-benzoquinone resulted in an increase in fungitoxicity, with no accompanying increase in phytotoxicity.

In an attempt to learn more about the reasons for this damaging action, we have prepared a number of compounds related to 2:3-dichloro-1:4-naphthaquinone

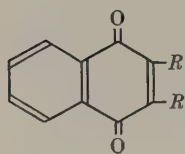
* Byrde, R. J. W., Crowdy, S. H. & Woodcock, D., 'Studies on Systemic Fungicides. III', *Ann. appl. Biol.* 40, 152, is regarded as Part I of this series.

and tested them for fungicidal efficiency and phytotoxic action. In a preliminary account already published (Byrde & Woodcock, 1952), we suggested that the high fungicidal activity of 1:4-diacetoxy-2:3-dichloronaphthalene in certain spore germination tests might result from breakdown products produced by fungal enzymes.

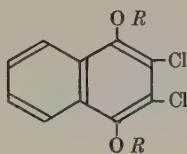
Read & Smith (1952) have subsequently suggested that the activity of the diacetyl derivative of 2:5-dichlorohydroquinone to two test fungi may also be due to hydrolysis of the ester by fungal spores. They attributed the relatively non-toxic nature of the diacetyl derivative of 2:3:5:6-tetrachlorohydroquinone to a less facile hydrolysis, owing to steric considerations. The diacetyl derivatives examined also proved less phytotoxic to tomato plants than the corresponding quinones.

A more detailed account of our earlier work, together with the results obtained using an extended series of esters of 2:3-dichloro-1:4-naphthahydroquinone, is presented below.

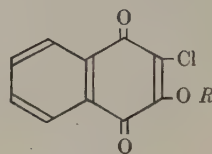
MATERIALS



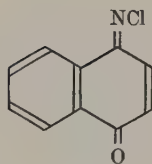
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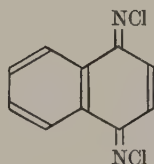
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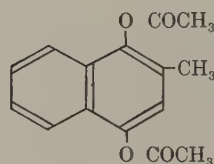
III



IV



V



VI

The 2:3-dichloro-1:4-naphthaquinone (I; R=Cl) used in this investigation was obtained from a commercial formulation by extraction with boiling chloroform and had m.p. 191–193° C.

All m.p.'s are uncorrected. C and H analyses are by Drs Weiler and Strauss, Oxford.

2:3-Dichloronaphthahydroquinone (II; R=H)

A solution of stannous chloride (16 g.) in warm concentrated hydrochloric acid (16 ml.) was added during 5 min. to a solution of 2:3-dichloro-1:4-naphthaquinone (8 g.) in boiling dioxan (20 ml.). After a further 5 min. heating charcoal was added and the solution filtered hot. On cooling the crystalline precipitate was collected, washed with water and dried at 100° C. It crystallized from aqueous methyl alcohol in monoclinic prisms, m.p. 155–156° C. (Found: C, 52.3; H, 2.9; Cl, 30.7. Calc. for $C_{10}H_6O_2Cl_2$; C, 52.4; H, 2.6; Cl, 31.0%). Graebe (1869) gives 135–140° C.; Claus (1886) gives 135° C.

1:4-Diacetoxy-2:3-dichloronaphthalene (II; R=COCH₃)

Acetylation of the above hydroquinone by refluxing with acetic anhydride and fused sodium acetate gave the di-acetyl derivative, which crystallized from acetone-ethyl alcohol in glistening monoclinic prisms, m.p. 239–240° C. (Found: C, 53.9; H, 3.5; Cl, 22.4. Calc. for C₁₄H₁₀O₄Cl₂: C, 53.7; H, 3.2; Cl, 22.7 %.) Graebe (1869) gives 236° C. According to Graebe, this compound is stable to boiling aqueous potassium hydroxide, but we found that after 2 hr. treatment with boiling 5 % sodium hydroxide, no original material was recoverable and the acidified solution was shown to contain acetic acid.

A *wettable powder* was prepared as follows: kaolin (3 parts) was added to a solution of the compound (1 part) in boiling acetone (100 vol.) and the solvent removed by distillation using an air stream to prevent settling of the carrier. The residual solid was dried at 70° C. and ground in a mortar.

1:4-Dipropionyloxy-2:3-dichloronaphthalene (II; R=CO.C₂H₅)

The dipropionic ester was prepared by refluxing with propionyl chloride in pyridine. It crystallized from ethyl alcohol in tiny needles, m.p. 166–167° C. (Found: C, 56.1; H, 4.16; Cl, 20.6. C₁₈H₁₄O₄Cl₂ requires C, 56.3; H, 4.10; Cl, 20.8 %.)

1:4-Dibutyryloxy-2:3-dichloronaphthalene (II; R=CO.C₃H₇)

Prepared using *n*-butyryl chloride in pyridine, the dibutyric ester crystallized from methyl alcohol in feathery prisms, m.p. 128–128.5° C. (Found: C, 58.6; H, 4.5; Cl, 19.3. C₁₈H₁₈O₄Cl₂ requires C, 58.5; H, 4.3; Cl, 19.2 %.)

1:4-Dibenzoyloxy-2:3-dichloronaphthalene (II; R=CO.C₆H₅)

Benzoylation of II (R=H) in pyridine gave the di-benzoyl ester which crystallized from acetone in small prisms, m.p. 252° C. (Found: C, 65.8; H, 3.3; Cl, 16.2. C₂₄H₁₄O₄Cl₂ requires C, 65.9; H, 3.2; Cl, 16.2 %.)

1:4-Dimethoxy-2:3-dichloronaphthalene (II; R=CH₃)

A solution of 2:3-dichloronaphthahydroquinone (2 g.) in anhydrous ether was cautiously added with cooling and shaking to a slight excess of diazomethane in ether. After standing overnight, the ethereal solution was washed with alkali and water and dried over anhydrous sodium sulphate. Removal of the solvent gave a product which crystallized from aqueous methyl alcohol in prismatic plates, m.p. 107–108° C. (Found: C, 56.4; H, 4.1; Cl, 27.1. C₁₂H₁₀O₂Cl₂ requires C, 56.0; H, 3.9; Cl, 27.6 %.)

3:4-Dichloro-1:2-naphthaquinone

Concentrated nitric acid (*d* 1.4, 2 ml.) was added dropwise during 15 min. to a stirred solution of 1:3:4-trichloro-2-naphthol (Fries & Schimmelschmidt, 1930) (0.5 g.) in glacial acetic acid (10 ml.). After stirring for a further 1 hr., the red crystalline precipitate was collected, washed with dilute acetic acid and water and dried at 100° C. This product crystallized from glacial acetic acid in red needles, m.p. 223–223.5° C. and is probably a *mono-nitro derivative*. (Found: Cl, 26.1. C₁₀H₃O₄NCl₂ requires Cl, 26.1 %.) Dilution of the acetic acid filtrate gave the quinone which crystallized from glacial acetic acid in red prismatic plates, m.p. 184–185°. (Found: Cl, 30.6. Calc. for C₁₀H₄O₂Cl₂: Cl, 31.3 %.) Zincke (1886) gives m.p. 184° C.

2-Chloro-3-acetoxy-1:4-naphthaquinone (III; R=COCH₃)

Prepared according to the method of Ullmann & Ettisch (1921) this compound crystallized from ethyl alcohol in golden yellow monoclinic prisms, m.p. 95–96° C. (Found: Cl, 14.4. Calc. for C₁₂H₇O₄Cl: Cl, 14.2 %.) Ullman & Ettisch give m.p. 98° C.

2-Chloro-3-hydroxy-1:4-naphthaquinone (III; R=H)

(a) Hydrolysis of the above acetyl compound by heating with 2% aqueous sodium hydroxide at 100° C. for 5 min., gave a product which crystallized from methyl alcohol in aggregates of rhombic prisms, m.p. 216–217° C. (Found: Cl, 16.7. Calc. for $C_{10}H_5O_3Cl$: Cl, 17.0%). Graebe (1869) gives m.p. 214–215° C.

(b) 2:3-Dichloro-1:4-naphthaquinone (1.0 g.) was refluxed with 5% sodium hydroxide solution for 2 hr. After cooling, acidification gave a yellow solid, m.p. 212–214° C., undepressed by admixture with the product from (a) above.

2:3-Dimethyl-1:4-naphthaquinone (I; R=CH₃)

Prepared according to the method of Fieser & Chang (1942) this crystallized from methyl alcohol in yellow monoclinic prisms, m.p. 127–128° C.

1:4-Naphthaquinone-N-chloroimide (IV)

4-Amino-1-naphthol hydrochloride (2.0 g.) was dissolved in water (100 ml.) by warming, concentrated hydrochloric acid (20 ml.) added and the solution stirred at 0° C. during the addition of a saturated solution of bleaching powder (50 ml.). The colour gradually changed, and a yellow precipitate which formed was filtered, washed with water and dried (2.4 g.). It crystallized from ethyl alcohol in golden yellow rectangular prisms, m.p. 111–111.5° C. (Found: Cl, 18.2. Calc. for $C_{10}H_6ONCl$: Cl, 18.5%). Friedländer & Reinhardt (1894) give m.p. 109.5° C. When a solution in glacial acetic acid was treated with excess concentrated hydrochloric acid at laboratory temperature the greyish green precipitate obtained after 10 min. was found to be 2:3-dichloro-1:4-naphthaquinone, since admixture with an authentic specimen failed to depress the m.p.

1:4-Naphthaquinone-NN'-dichloroimide (V)

The preparation of this compound from 1:4-naphthalene diamine, as described by Böchmann & Friedländer (1889), was found to be difficult and erratic and the yields from a number of attempts proved minute.

In some experiments the product crystallized from the reaction mixture in tiny yellowish needles, m.p. 136–137° C. (Found: Cl, 31.4. Calc. for $C_{10}H_2N_2Cl_2$: Cl, 31.6%). Recrystallization from ethyl alcohol usually led to 2:3-dichloro-1:4-naphthaquinone.

1:4-Diacetoxy-2-methylnaphthalene (VI)

We are indebted to Messrs Ward Blenkinsop for a gift of 'Acetomenaphthone', which was used as received, m.p. 113–114° C.

METHODS

Determination of rates of hydrolysis

Hydrolysis of these esters was made difficult by their water-repellent nature. For this reason it was not found possible to get concordant results by doing a continuous hydrolysis run and removing aliquots at intervals. Accordingly, hydrolysis was carried out by refluxing the compound (0.20–0.22 g.) with N/1 sodium hydroxide (5 ml.) for standard times (0.25, 0.5, 1.0, 2.0, 4.0 hr.). After acidifying with 50% sulphuric acid (0.6 ml.) the volatile fatty acid in the mixture was estimated by a modification of Hillig & Knudsen's (1942) method.

In vitro fungitoxicity

A test-tube dilution spore germination technique, based on that recommended by the American Phytopathological Society, Committee on Standardization of Fungicidal Tests (1943, 1947), was used for the evaluation of fungistatic activity *in vitro*. Four test fungi were studied.

Spores were obtained as follows:

Sclerotinia laxa Aderh. & Ruhl.: from 7-day-old cultures grown on autoclaved potato plugs previously soaked in 7.5 % malic acid for 72 hr.

Botrytis fabae Sardiña: from 7-day-old cultures grown on a liquid medium of the following composition:

Dextrose	30 g.	Potassium dihydrogen phosphate	5 g.
Peptone	10 g.	Ferric citrate	0.02 g.
Potassium nitrate	2 g.	Calcium chloride	0.01 g.
Magnesium sulphate (7H ₂ O)	1 g.	Distilled water to	1 l.

This medium was developed by Mr A. H. Fielding, and was found to give high spore yields.

Cladosporium fulvum Cooke: from lesions on infected tomato leaves.

Venturia inaequalis (Cooke) Wint.: the earlier tests were made with spores from infected apple leaves. In later trials, spores were obtained from autoclaved twigs of apple stocks by a method developed by Montgomery & Moore (1937), and modified by Kirkham & Frick (1952).

In each case, suspensions were prepared by gently washing off the spores from the substrate with distilled water. After centrifuging for 30 sec. at 1700 r.p.m. the spores were suspended in distilled water, and their concentration adjusted to 500,000/ml. by means of a haematocytometer cell.

The compounds under test, which were virtually insoluble in water, were dissolved in acetone at a concentration of 1000 p.p.m. For preliminary testing, the acetone solution (0.5 ml.) was run into distilled water (100 ml.) with vigorous agitation. A sample of the resulting suspension (0.8 ml.) was pipetted into a small test-tube (5 × 1 cm.), and to it was added 0.2 ml. of a mixture of equal parts of a spore suspension of the appropriate fungus (500,000/ml.) and a spore stimulant consisting of 1 % sucrose and 0.01 % sodium citrate. The final composition of the system was thus:

Fungicide	4 p.p.m.	Sucrose	0.1 %
Acetone	0.4 %	Sodium citrate	0.001 %
Spores	50,000/ml.		

After thorough agitation, two drops of the mixture were pipetted out on cellulosed glass slides. The germination of fifty spores in each drop was recorded after incubation at 25° C. for 17 hr. in a moist chamber of 6 in. diameter. Controls were included; the presence of 0.4 % acetone in the drops at the time of pipetting had no effect on germination.

When a compound merited further examination, a series of dosages was prepared and tested as above. Dosage-mortality data were mathematically computed from the percentage germination figures by conventional methods (Finney, 1947). For more satisfactory comparison of derivatives, concentrations were expressed on a molar basis.

One fungicide was also tested as a 25 % wettable powder on kaolin and as a suspension with an equal concentration of sulphite lye.

The methods used in phytotoxicity and manometric experiments are noted briefly under these respective headings.

RESULTS

Ester hydrolysis

Fig. 1 illustrates the different rates of alkaline hydrolysis of four esters of 2:3-dichloro-1:4-naphthahydroquinone. As expected, no hydrolysis of the methyl ether was observed.

In vitro fungitoxicity

2:3-Dichloro-1:4-naphthahydroquinone, derived esters and methyl ether

The following compounds proved virtually inactive against all four fungi in preliminary tests at 4 p.p.m., and were not tested further: 2:3-dichloro-1:4-dimethoxynaphthalene, 1:4-dibenzoyloxy-2:3-dichloronaphthalene.

Table 1 summarizes the results (expressed on a molar basis) of more detailed tests of the remainder, using 2:3-dichloro-1:4-naphthahydroquinone as a standard.

1:4-Diacetoxy-2:3-dichloronaphthalene was further tested against *V. inaequalis* in the presence, and in the absence, of a small quantity of a commercial preparation of a fungal pectic enzyme known to possess high esterase activity towards phenyl acetate. Fig. 2 illustrates the results obtained. It will be seen that the fungicidal activity of the diacetyl derivative towards *V. inaequalis* was markedly increased by the addition of the enzyme preparation. The latter was found to have no fungistatic activity *per se*.

Formulation of 1:4-diacetoxy-2:3-dichloronaphthalene

The effect of different formulations of the diacetyl derivative on its fungicidal activity towards *Sclerotinia laxa* is illustrated by Fig. 3. The dosage-response lines for the aqueous preparations were parallel, the kaolin preparation being the more effective. The acetone formulation was still more active, with a steeper dosage-response line.

Particle size determinations were carried out on each formulation, and are quoted in the caption to Fig. 3.

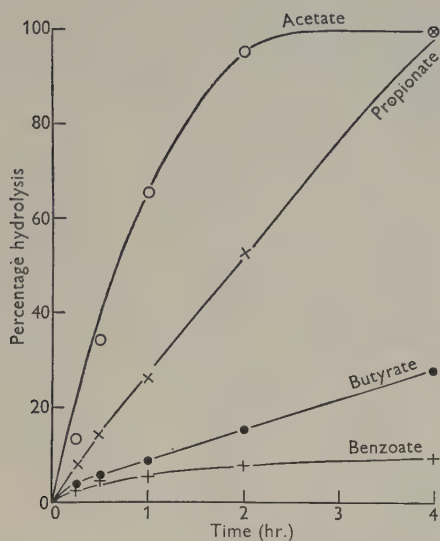


Fig. 1. Alkaline hydrolysis rates of esters of 2:3-dichloro-1:4-naphthahydroquinone.

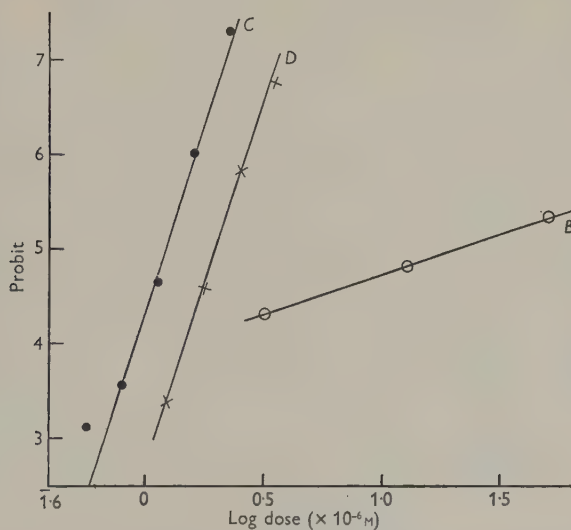


Fig. 2. The effect of an added source of acetylcholinesterase on the fungicidal activity of 1:4-diacetoxy-2:3-dichloronaphthalene against conidia of *V. inaequalis*. B, 1:4-diacetoxy-2:3-dichloronaphthalene alone; C, 1:4-diacetoxy-2:3-dichloronaphthalene plus added enzyme; D, 2:3-dichloro-1:4-naphthaquinone (standard).

TABLE 1. Dosage-response data: 2:3-dichloro-1:4-naphthalhydroquinone and its alkyl esters tested against four fungi, using 2:3-dichloro-1:4-naphthaquinone as standard

Compound	<i>Sclerotinia laxa</i>			<i>Botrytis fabae</i>			<i>Cladosporium fulvum</i>			<i>Venturia inaequalis</i>		
	Equation of line	ED 50 ($\times 10^{-6}$ M)	Relative potency*	Equation of line	ED 50 ($\times 10^{-6}$ M)	Relative potency*	Equation of line	ED 50 ($\times 10^{-6}$ M)	Relative potency*	Equation of line	ED 50 ($\times 10^{-6}$ M)	Relative potency*
2:3-dichloro-1:4-naphthalhydroquinone	$Y=6.90x+5.37$	0.88	1.01 (0.94-1.09)	$Y=15.44x-2.90$	3.25	1.02 (0.97-1.07)	$Y=12.06x+1.80$	1.84	1.00 (0.95-1.06)	$Y=6.17x+8.94$	0.23	1.23 (1.13-1.34)
2:3-dichloro-1:4-naphthaquinone	$Y=6.90x+5.33$	0.90	1.00	$Y=15.44x-3.04$	3.32	1.00	$Y=12.06x+1.79$	1.84	1.00	$Y=6.17x+8.39$	0.28	1.00
1:4-diacetoxy-2:3-dichloronaphthalene	$Y=7.30x+4.82$	1.06	0.99 (0.92-1.07)	$Y=6.76x+4.69$	1.11	2.09 (1.92-2.27)	$Y=4.92x+4.97$	1.02	1.13 (1.00-1.27)	$Y=0.86x+3.88$	20.14	†
2:3-dichloro-1:4-naphthaquinone	$Y=7.30x+4.84$	1.05	1.00	$Y=6.76x+2.52$	2.32	1.00	$Y=4.92x+4.71$	1.15	1.00	$Y=7.83x+2.69$	1.97	†
1:4-dipropionyloxy-2:3-dichloronaphthalene	$Y=8.17x+4.54$	1.14	0.75 (0.67-0.83)	$Y=7.26x+2.08$	2.52	0.79 (0.71-0.88)	—	—	—	—	—	—
2:3-dichloro-1:4-naphthaquinone	$Y=8.17x+5.58$	0.85	1.00	$Y=7.26x+2.84$	1.98	1.00	—	—	—	—	—	—
1:4-dibutyryloxy-2:3-dichloronaphthalene	—	—	—	$Y=4.59x+2.73$	3.13	0.54 (0.45-0.65)	—	—	—	—	—	—
2:3-dichloro-1:4-naphthaquinone	—	—	—	$Y=4.59x+3.95$	1.70	1.00	—	—	—	—	—	—

* 5 % Fiducial limits are stated in brackets.

† Dosage-response lines not parallel.

Results not quoted indicate low fungicidal activity in the preliminary test at 4 p.p.m.

Miscellaneous naphthaquinone derivatives

The following compounds proved virtually inactive against conidia of *S. laxa* at 4 p.p.m. and were not further tested: 2-chloro-3-hydroxy-1:4-naphthaquinone, 1:4-diacetoxy-2-methyl-naphthalene, 2:3-dimethyl-1:4-naphthaquinone.

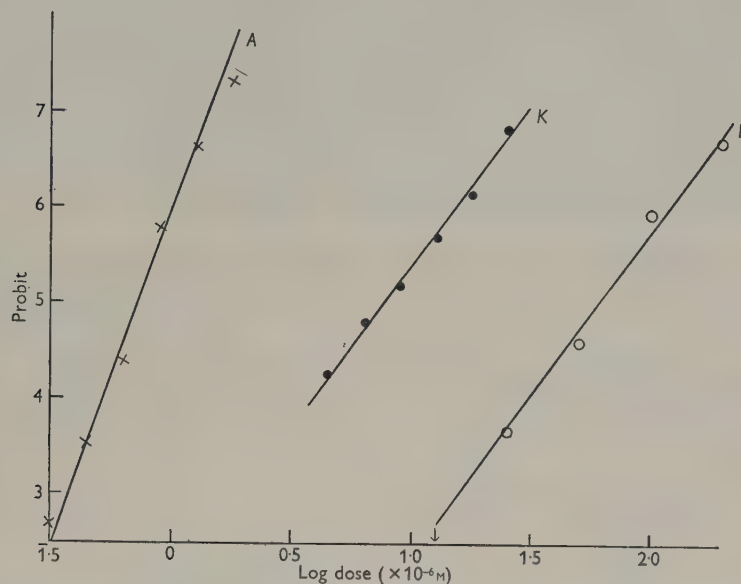


Fig. 3. The effect of formulation on fungicidal activity of 1:4-diacetoxy-2:3-dichloro-naphthalene. *A*, in aqueous acetone (average particle size $1.83 \pm 1.43 \mu$); *K*, as 25 % wettable powder on kaolin (average particle size $3.46 \pm 2.84 \mu$); *L*, as sulphite lye suspension (average particle size $12.28 \pm 10.72 \times 2.88 \pm 1.43 \mu$).

TABLE 2. *Dosage-response data: miscellaneous naphthaquinone derivatives tested against Sclerotinia laxa using 2:3-dichloro-1:4-naphthaquinone as standard*

Compound	Equation of line	ED ₅₀ ($\times 10^{-6} M$)	Relative potency	5 % fiducial limits
3:4-dichloro-1:2-naphthaquinone	$Y = 7.64x - 1.53$	7.16	0.10	0.09-0.10
2:3-dichloro-1:4-naphthaquinone	$Y = 7.64x + 6.23$	0.69	1.00	—
2-chloro-3-acetoxy-1:4-naphthaquinone	$Y = 4.96x + 2.04$	3.95	0.16	0.14-0.18
2:3-dichloro-1:4-naphthaquinone	$Y = 4.96x + 6.01$	0.62	1.00	—
1:4-naphthaquinone- <i>N</i> -chloroimide	$Y = 10.08x - 3.92$	7.67	0.15	0.14-0.16
1:4-naphthaquinone- <i>NN'</i> -dichloroimide	$Y = 10.08x - 2.20$	5.17	0.22	0.21-0.24
2:3-dichloro-1:4-naphthaquinone	$Y = 10.08x + 4.38$	1.15	1.00	—

Table 2 shows the results (expressed on a molar basis) of a more detailed study of the activity of four other compounds proving effective against conidia of *S. laxa* at 4 p.p.m.

Phytotoxicity as foliage and fruit sprays

The relative phytotoxicities of 2:3-dichloro-1:4-naphthaquinone and two derivatives of high *in vitro* fungicidal activity were tested by their effects on young potted plants of tomato (var. Market King) and broad bean (var. Giant Windsor) in a greenhouse, and on fruitlets of plum (var. Victoria) and apple (var. Worcester Pearmain) in the orchard. The materials were each dissolved in acetone at 0.5 %, and the acetone solution diluted with water containing 0.25 % of a proprietary wetting and dispersing agent. Sprays were applied by means of a small hand atomizer in the greenhouse, and with a bucket-type sprayer in the orchard.

The degree of damage was assessed on an arbitrary scale ranging from 0 (nil) to 3 (severe). Table 3 summarizes the results of duplicate tests.

TABLE 3. *Phytotoxicity of 2:3-dichloro-1:4-naphthaquinone and two derivatives*

Compound	Conc. (%)	Mean degree of damage		
		Bean	Tomato	Plum
2:3-dichloro-1:4-naphthaquinone	0.1	0.5	0.5	—
	0.05	—	—	2.0
2:3-dichloro-1:4-naphthahydroquinone	0.1*	0.5	1.0	—
	0.05*	—	—	2.0
1:4-diacetoxy-2:3-dichloronaphthalene	0.14*	0.0	0.0	—
	0.07*	—	—	0.0

* These concentrations are comparable on a molar basis with those of the parent quinone.

The absence of damage by the diacetyl derivative is noteworthy. No damage from any of the three sprays was encountered on leaves or fruitlets of apple.

Manometric studies (with A. H. FIELDING)

Attempts were made to study the enzymic hydrolysis of 1:4-diacetoxy-2:3-dichloronaphthalene by means of a Warburg micro-respirometer. A partially purified preparation from macerated mycelium of *S. laxa* was used as a source of the enzyme, and its activity was measured at pH 7.0 by the liberation of carbon dioxide from a solution of sodium bicarbonate (0.03 M) in a N₂/CO₂ atmosphere. Under these conditions such an enzyme preparation showed high esterase activity towards phenyl acetate.

The rate of hydrolysis of 1:4-diacetoxy-2:3-dichloronaphthalene in the presence of 5 % acetone was found to be less than 0.2 % of that of phenyl acetate; such a rate of hydrolysis was found to be too low to determine accurately. No measurable hydrolysis of the diacetyl ester was detected in the case of the wettable powder preparation. 1:4-Diacetoxy-2-methylnaphthaquinone was hydrolysed at a rate of 45 % by comparison with phenyl acetate. A full report of these manometric studies will be published elsewhere.

DISCUSSION

The biological activity of quinones has been variously attributed to blocking of vital enzymes by substitution or addition at the double bond of the quinone nucleus, an interference with a cell redox system or an oxidative effect on sulphhydryl enzymes (McNew & Burchfield, 1951).

Two significant results were noted in a preliminary report on the examination of the fungicidal activity of certain 2:3-dichloro-1:4-naphthaquinone derivatives (Byrde & Woodcock, 1952). First, reduction of the naphthaquinone to the corresponding hydroquinone, followed by acetylation, produced a compound with reduced phytotoxicity, yet of the same order of activity in spore germination tests against *S. laxa*, *Botrytis fabae* and *Cladosporium fulvum*. Furthermore, parallel dosage-response lines were obtained for each organism suggesting a similar mode of action.

If the action of a quinonoid type fungicide is dependent on the presence of the carbonyl groups, then the activity of the esters will depend on their hydrolysis to the

TABLE 4. *Comparison of hydrolysis rates of derivatives of 2:3-dichloro-1:4-naphtha-hydroquinone and their activity against Botrytis fabae relative to 2:3-dichloro-1:4-naphthaquinone*

Derivative	Percentage hydrolysis after 2 hr.*	Relative potency
Diacetyl ester	96	2.09
Dipropionyl ester	52	0.79
Dibutyl ester	16	0.54
Dibenzoyl ester	8	Very low
Dimethyl ether	0	Very low

* Derived from fig. 1.

hydroquinone which could then participate in an oxidation-reduction system. The toxicity of such an ester to a given fungus should therefore bear some relationship both to the ease with which it can be hydrolysed and to the availability of a suitable fungal esterase. The necessity for both these conditions is apparent from the experimental evidence presented in this paper. Thus a close relationship appears to exist between the fungicidal activity and the rates of hydrolysis which are compared in Table 4.

Moreover, in an experiment using *Venturia inaequalis*, against which the diacetyl ester was relatively ineffective, the addition of an enzyme preparation exhibiting high acetyl-esterase activity markedly increased the susceptibility of the fungus.† The influence of the ease of hydrolysis of hydroquinone esters on their fungitoxicity has also been emphasized by Read & Smith (1952).

† *Note added in proof.* This result is closely analogous to the increased antibacterial action of the diphosphates of 1:4-dihydroxynaphthalene and 2-methyl-1:4-dihydroxynaphthalene following treatment with alkaline phosphatase (Marrian, Friedmann & Ward, 1953).

The rate of enzymic hydrolysis of the 1:4-diacetoxy-2:3-dichloronaphthalene was too low for effective measurement by the Warburg micro-respirometer. It is significant, however, that the lowest rate which could be accurately measured would result in a fungitoxic concentration of hydrolysis product after a period of only 15 min. An example of the facile hydrolysis of an ester is afforded by 1:4-diacetoxy-2-methyl naphthalene which was broken down at approximately one-half the rate at which phenyl acetate could be hydrolysed. This compound, which proved virtually inactive at 4 p.p.m. against *S. laxa*, was also found by Horsfall (1945, p. 146) to be non-toxic. He concluded that since the parent 2-methyl-1:4-naphthaquinone has some activity, being stronger than 1:4-naphthaquinone itself, this loss of toxicity following reduction and acetylation must be due to destruction of the quinonoid structure. This view is contrary to the hypothesis which we have outlined to explain the activity of the non-quinonoid 1:4-diacetoxy-2:3-dichloronaphthalene. Horsfall did not specify the fungus used, however, and the possibility exists that esterase secretion was inadequate.

That particle size affects the activity of 1:4-diacetoxy-2:3-dichloronaphthalene appears evident from Fig. 2. The importance of this effect on field performance of 2:3-dichloro-1:4-naphthaquinone has already been proved by McNew & Burchfield (1950), who demonstrated a direct correlation between its effectiveness in protecting foliage, and the particle size at the time of deposition. These authors have also stressed the consequent difficulty of designing a reliable spore-germination technique and suggested precipitation of the fungicide from a water-miscible solvent. Most of our tests were carried out by this method, but it became evident that such a technique tended to 'flatter' the performance of the fungicide by comparison with a formulation suitable for field application.

The lack of phytotoxicity of the esters of 2:3-dichloro-1:4-naphthahydroquinone may be ascribed to their inherent inactivity. Breakdown to an active material would take place only in the presence of a suitable fungal spore, and the liberation of toxic principle would occur only in its immediate vicinity. Such a concept may well have wider application in plant protection.

The necessity for the presence of the two vicinal chlorine atoms in the 1:4-naphthaquinone nucleus for high fungicidal activity is emphasized by the inactivity of the isosteric compounds 2:3-dimethyl- and 2-chloro-3-hydroxy-1:4-naphthaquinone. The effect of the presence of the *N*-chloroimide group was also examined but both the *N*-chloro- and the *NN'*-dichloroimides of 1:4-naphthaquinone showed markedly less activity than 2:3-dichloro-1:4-naphthaquinone. The isomeric 3:4-dichloro-1:2-naphthaquinone was also appreciably less active.

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TOXICITY TO THE GRAIN WEEVIL OF SOME ALKYL COMPOUNDS APPLIED AS VAPOURS

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(With 3 Text-figures)

A simple fumigation technique is described, which allows twenty to thirty tests to be made at once with different concentrations of vapour. The 50 % lethal doses (LD 50's) of different substances were estimated by the probit method of analysis, the slopes of the probit lines also being recorded.

The present work had two aims, the first being the investigation of the relationship of the molar LD 50's of different substances to one another. The substances were so chosen that comparison could be made both within homologous series and, for compounds with the same alkyl groups, between them. The second aim was to find out to what extent the slopes of probit lines varied for compounds believed to have like and unlike modes of action.

Some of the series were known to contain substances with an anaesthetic or narcotic effect, symptoms which are frequently attributed to substances with some type of physical action. Other series included compounds which had little or no observed narcotic effect, which were lethal at low vapour concentrations and for which evidence exists that they possess chemical toxicity for some organisms. A series which might reasonably be expected to have some of the attributes of both the 'physical' and chemical toxicants was also used.

On plotting log molar LD 50's against the number of alkyl carbon atoms, the well-known linear relationship was noted for the series with an observed narcotic effect, the constant logarithmic difference being large in all except the alcohol series. However, the upper members of the series which were presumed chemically toxic also possessed toxicities which showed a constant logarithmic difference, but a much smaller one than that characteristic of the first group. The bromide series had for its higher members a constant difference in the log LD 50's intermediate between these two groups. On the other hand, the lower members of the bromide, iodide and thiocyanate series had log LD 50's which were not related in any obvious way. These experimental results are discussed in terms of phase distribution and chemical reactivity.

Two poisons whose modes of action on a given organism are much alike often show parallel probit regression lines, especially if they possess similar chemical constitutions. There is, however, no reason why this should necessarily be the case, and in the present investigation it was found that compounds with an observed anaesthetic or narcotic effect often had very different probit slopes. Within most series, the slope was not greatly different except for the lower and higher members, for which it often appeared higher than for the intermediate ones. Between series, a rough correlation often existed between probit slope and median response vapour pressure.

INTRODUCTION

The following paper reports the results of investigations into the toxicity to the grain weevil, *Calandra granaria* L., of twenty-nine organic substances. With the exception of chloroform, all are normal members of six relatively simple homologous series, some of which contain substances which are often stated to possess a physical action only. Each series has been studied from the lowest member for which the experimental technique was applicable, up to the cut-off point, so that comparison of toxicity is possible both within and across series, and for both external toxic concentration and the relative saturation of toxic action. It is hoped that this systematic but limited study will provide further information about the way in which drug action is related to the physical properties and the chemical reactivity of toxicants.

Wherever possible, experimental data were subjected to statistical analysis by the probit method. It was evident that the slopes of probit graphs, and sometimes apparently their shapes also, differed quite markedly, and no attempt has been made to apply the constraint of parallelism even to graphs of closely allied substances.

EXPERIMENTAL METHODS

Biological and statistical

One-litre conical flasks were one-third filled with wheat grain, plugged with cotton-wool, and steam sterilized. The original stock culture of *C. granaria* was obtained from the Pest Infestation Laboratory at Slough. The flasks were inoculated at suitable intervals with about 100 1 week-old grain weevils from previous cultures, the cotton-wool plugs being replaced by covers of several layers of muslin, held taut by rubber bands. The inoculated flasks were stored in an incubator at 25°C. for 5 weeks, after which the adult beetles were sieved off and rejected. The sieve, of 2 mm. mesh, was large enough to allow weevils to fall through while the wheat grains, containing eggs and larvae, remained on top. Sieving was repeated 10 days later, after the young beetles had started to emerge, and thereafter at weekly intervals for 3 weeks. The beetles from different sievings were therefore of comparable age, and were used for toxicity tests 3–6 days after sieving. Busvine (1938) considered that relative humidity was of little importance in determining changes in beetle resistance, and no humidity control was included in the incubator in which the beetle cultures were placed. However, as a precautionary measure, the flasks containing the sieved progeny were put into a desiccator, containing a little water, used as a constant humidity chamber. The desiccator was then placed in the incubator. It was advantageous to add, as food, a few grains of wheat to the flasks of sieved beetles since this was found to minimize mortality of controls during the tests.

Prior to a toxicity test, the beetles were tipped on to a piece of glazed paper, and brushed on to another piece of paper as they walked away from the light. By this means, batches of twenty were placed in weighing bottles. When sufficient had been

counted out for the experiment, the contents of two bottles, randomly selected, were combined. This latter procedure for obtaining batches of forty beetles was adopted so as to reduce the risk of non-uniform sampling arising out of the fact that the stronger or more agile beetles tended to walk off the paper sooner than the remainder.

The use of several types of fumigation apparatus was investigated, but the results reported in this paper were all obtained from the simplest, based on stoppered 500 ml. 'Quickfit' flasks. Flasks where substances were volatilized *in vacuo* were not found to be superior in practice to those used, and were more difficult to handle, while any apparatus containing rubber stoppers gave most unreliable results.

Generally, fifteen or more flasks were used in each experiment, and two or more experiments were carried out to obtain probit regression data for one substance. The method of measuring and introducing the toxicant depended upon the magnitude of the external toxic concentration. For some hydrocarbons, alkyl chlorides and bromides, and chloroform, it was possible to measure the substance in a semi-micro-pipette; where smaller quantities were lethal, a glass phial containing the liquid was placed in the flask. In the first case, the stopper was replaced immediately the substance had been added. In the second, the phial was first carefully crushed using a glass plunger fitted into a detachable handle. The plunger was thus left in the flask when the stopper was replaced. (These glass phials which are rapidly made from drawn-out glass tubing, were $\frac{1}{4}$ – $\frac{1}{2}$ in. long, sealed at one end, and drawn out at the other to a neck at right angles to the phial. They were weighed, warmed, the liquid toxicant being drawn in on cooling, and then sealed and re-weighed.) Whichever method was used, the flasks were incubated for an hour at 25° C. to allow the liquid to volatilize completely.

Meanwhile, the batches of forty beetles were emptied into small, stoppered ignition tubes around the necks of which pieces of cotton were tied, and each tube was provided with a small piece of wire gauze upon which the beetles could climb. The tubes containing the beetles were placed for about 45 min. in the same darkened incubator as the fumigation flasks, to allow the beetles to recover from the counting and transfer. The stoppers were then removed from the ignition tubes, and each tube was suspended quickly within a fumigation flask by means of the cotton thread. The fumigation continued 5 hr. at 25° C. in a darkened cupboard, the flasks being gently shaken every half an hour to keep the vapour evenly distributed. At the end of the exposure time, the tubes containing the beetles were removed, the weevils tipped into labelled weighing bottles, each containing two grains of wheat, and secured with a folded muslin cover. The bottles were then put into the humidity chamber in an incubator at 25° C. and left for 4 days, after which the numbers of weevils alive, dead and moribund were determined. Four days was the time interval between exposure and counting which was found by trial and error to give optimum results; after this time deaths amongst the control organisms were more common. For computational purposes, the moribund beetles were

regarded as 'half-dead' in order to avoid attaching too much weight to these figures, although generally less than 10% of the organisms fell within this class. The moribund beetles were arbitrarily considered to be those which were able to move slightly, but were unable to walk off a 4 in. square paper in 4 min. Where a small percentage of the control beetles died, allowance was made for this, using Abbott's correction.

The two chief difficulties of using this technique are related to the opening of the flasks to introduce the beetles, and to the fact that the experiments were all carried out at atmospheric pressure. The first of these can be overcome by allowing a correction factor for the volume of air-plus-vapour lost on opening the flasks. It is relatively small, and can be accounted for by regarding the effective volume of the flask as being about 10 ml. more than its measured volume. Moreover, for the purposes of this study, relative values for the substances were more important than the absolute toxic concentrations, and any error in the estimation of this correction factor will apply equally to all results. Secondly, for a few of the less toxic materials, considerable quantities of substance were necessary to effect 50% kill, and the vapour after the evaporation of the liquid exerted a pressure within the flask. Vapour and air therefore escaped on introducing the weevils. Knowing the weight of substance introduced, the volume of air-plus-vapour escaping could be calculated from the gram-molecular volume, and the effective concentration in each flask corrected accordingly.

The primary statistical analysis was carried out using the probit method of Finney (1952). A line was drawn through the plotted points by eye, and the line of best fit determined from it. Generally, only one cycle of calculations was necessary. Sometimes the points were randomly distributed around this straight line, whereas in other cases points tended to fall above the line at the ends and below it in the middle, suggesting that the probit line might in fact be curved. In these latter instances, lower, median and upper regression lines were also fitted to the points, again using the probit method. The arbitrary limits defining the central region were the probits 4.3 and 5.7. For the last effectively toxic member of some series, where only about 50% kill was obtained using the saturated vapour, the probit method is not applicable (Ferguson & Pirie, 1948), and a calculated regression line was not fitted. For these substances, an estimate of the LD 50 was obtained by extrapolation of a line or curve fitted by eye.

Chemical

The alkyl thiocyanates were synthesized from alkyl iodides and sodium thiocyanate using alcohol as solvent. Bromides and iodides, where necessary, were prepared from alcohols, red phosphorus and the halogen. All the substances used, except for a few chlorides, were purified by vacuum distillation. As a result of this process, data were obtained from which Clausius-Clapeyron graphs could be drawn; these proved valuable in that they allowed an estimate of saturation vapour

pressures at 25° C. to be made for substances for which no data were available in the literature. Boiling-point data for the thiocyanates are presented in Table 1.

RESULTS

Concentrations causing 50% kill

The LD 50's were determined as log mg./l. from the straight probit lines of best fit. The calculated standard errors of these figures were in general only about 2% of the dosage values. Such standard errors are misleadingly small, however, since the experiments were not all carried out at one time on one set of beetles, and batch-to-batch variation in sensitivity may have occurred. Substances within each series were all tested within a few days of one another, and hence the disturbing effect of any such change was minimized. Moreover, the LD 50's which have been recorded were all calculated from data obtained in several experiments and except occasionally for members near the cut-off point, no great lateral shift in the log tolerances was observed. At 25° C., the relationship

$$\log p_t + \log \text{mol. wt.} - 1.2693 = \log m$$

enables the value of p_t , and therefore of the relative saturation, p_t/p_s , to be determined. In the equation, p_t is the median response vapour pressure and m is the toxic concentration expressed as mg./l. The saturation vapour pressures at the same tem-

TABLE 1. *Boiling-point data for the normal thiocyanates*

Substance	Boiling point at		
	50 mm.	100 mm.	760 mm.
Methyl thiocyanate	54.1	69.2	130.8
Ethyl thiocyanate	65.5	83.2	143.9
Propyl thiocyanate	81.9	100.2	165.3
Butyl thiocyanate	~98.4	127.1	185.2
Amyl thiocyanate	~114.3	~133.9	204.7

perature, p_s , were obtained from the literature, or were determined from Clausius-Clapeyron data after applying an empirical correction to allow for superheating. The values for butyl and amyl thiocyanate are only approximate, owing to the relatively large correction factors for such low vapour pressures. In the final column of Table 2 the substances have been divided into physical, chemical and 'physico-chemical' poisons, a procedure first adopted by Ferguson & Pirie (1948). Here, however, the classification is based more upon the way in which the log molar LD 50's for succeeding homologues are related to one another than upon the absolute magnitude of the relative saturation value corresponding to each toxic dose.

Shapes and slopes of probit lines

The slopes of the probit-log mg./l. general regression lines together with their variances, appear in Table 3. On transformation to log molar concentrations, the positions but not the slopes of these lines change. The unweighted mean value of

$Y(\bar{y})$, which is of importance in the cases where the probit line is apparently curved, is also recorded for each set of data. For curved lines no one regression line adequately fits all the points, and as mentioned above, lower, median and upper regression lines have been calculated in addition to the general line which has been used to determine lethal dosages. Since the lines for lower log concentrations have the lower slopes, if

TABLE 2. *Toxicity test results. Lethal doses for 5 hr. exposure*

Substance	Log m_{50} (mg./l.)	Log mol./l. ($\times 10^4$) ($Y=5.0$)	p_s (mm.)	p_s/p_s ($Y=5.0$)	Type
Pentane	2.8842	2.026	50.1	0.39	P
Hexane	2.5840	1.648	143	0.58	P
Heptane	2.3617	1.361	45.7	0.93	P
Octane	~ 2.000	0.943	15.9	> 1.00	P
Methyl alcohol	1.9671	1.462	135.6	0.40	P or PC
Ethyl alcohol	1.9785	1.315	63.3	0.64	P or PC
Propyl alcohol	1.9646	1.186	23.6	> 1.00	P or PC
Chloroform	2.2904	1.214	199	0.15	P
Propyl chloride	2.6928	1.798	339	0.34	P
Butyl chloride	2.3803	1.414	107	0.45	P
Amyl chloride	1.9590	0.932	32	0.50	P
Hexyl chloride	1.6767	0.595	10	0.73	P
Ethyl bromide	2.0633	1.026	468	0.042	C
Propyl bromide	2.0230	0.933	135	0.12	C
Butyl bromide	2.0200	0.883	38.9	0.37	PC
Amyl bromide	1.7344	0.556	13.8	0.49	PC
Hexyl bromide	1.5255	0.307	3.97	0.95	PC
Methyl iodide	0.2490	1.097	402	0.00058	C
Ethyl iodide	0.9549	1.762	138.8	0.0077	C
Propyl iodide	0.8088	1.579	43.2	0.016	C
Butyl iodide	0.8891	1.624	12.9	0.061	PC
Amyl iodide	0.8169	1.520	4.14	1.15	PC
Hexyl iodide	0.7148	1.389	1.15	0.40	PC
Heptyl iodide	0.5958	1.240	~ 0.35	0.84	PC
Methyl thiocyanate	0.6159	1.752	10.0	0.11	C
Ethyl thiocyanate	0.8216	1.881	4.50	0.31	PC
Propyl thiocyanate	0.6698	1.665	2.01	0.43	PC
Butyl thiocyanate	0.6097	1.548	0.79	0.83	PC
Amyl thiocyanate	~ 0.455	1.344	~ 0.35	> 1.00	PC

Y =probit; P=physical; C=chemical; PC=physico-chemical; p_s =saturation vapour pressure at 25° C.

\bar{y} is less than 5 the observed general regression slope will be weighted below its true value, and vice versa for \bar{y} values of greater than 5. The slopes of the lower, median and upper lines are recorded in Table 4, and the deviation from linearity for the general line may be assessed from the relative magnitudes of these values.

Observed physiological effect

Two types of response to toxicants were noted, with a gradation of response between them. At one extreme, anaesthetic substances caused complete loss of mobility which often continued many hours, and occasionally even for 1-2 days,

after exposure to the vapour. This situation occurred for compounds such as propyl chloride, chloroform and pentane, which have LD 50's corresponding to moderately low p_i/p_s values, and which have often been considered to have, at least primarily, a physical action. The beetles which recovered from the initial effect mostly remained alive for the rest of the 4-day period before counting. At the other

TABLE 3. *Slopes of general probit regression lines*

Substance	Slope, b (5 hr. test)	V_b	n	\bar{y}	Type of graph
Pentane	14.5	0.85	21	5.5	Linear
Hexane	11.0	0.55	31	5.0	Linear
Heptane	13.1	0.66	33	4.6	Linear
Octane	—	—	35	—	None
Methyl alcohol	9.14	0.14	45	5.2	Linear
Ethyl alcohol	9.48	0.35	26	4.7	Linear
Propyl alcohol	—	—	30	3.8	None
Chloroform	10.3	0.57	17	5.4	Linear
Propyl chloride	19.7	0.64	48	5.2	Linear
Butyl chloride	7.53	0.20	34	4.9	Linear
Amyl chloride	7.88	0.26	22	4.9	Linear
Hexyl chloride	8.17	0.33	15	4.7	Linear
Ethyl bromide	12.4	0.44	26	5.3	Linear
Propyl bromide	9.73	0.19	43	5.0	Curved
Butyl bromide	7.94	0.27	31	4.5	Slightly curved?
Amyl bromide	8.40	0.24	26	4.7	Linear
Hexyl bromide	10.9	1.36	22	4.0	Linear?
Methyl iodide	—	—	7	6.0	Linear?
Ethyl iodide	5.63	0.107	21	5.4	Slightly curved?
Propyl iodide	4.34	0.050	25	5.5	Linear
Butyl iodide	5.21	0.060	36	4.9	Slightly curved?
Amyl iodide	4.02	0.046	24	5.3	Linear
Hexyl iodide	6.50	0.089	34	5.0	Linear
Heptyl iodide	10.6	0.608	18	4.5	Linear
Methyl thiocyanate	6.26	0.126	29	5.4	Curved
Ethyl thiocyanate	5.27	0.110	31	4.8	Curved
Propyl thiocyanate	6.78	0.140	32	5.4	Slightly curved
Butyl thiocyanate	7.49	0.190	33	5.0	Linear
Amyl thiocyanate	—	—	25	—	None

n =number of flasks, each containing forty beetles; V_b =variance of the slope; \bar{y} =unweighted mean probit value.

TABLE 4. *Slopes of lower, median and upper probit-log mg./l. regression lines*

Substance	Slope of regression line		
	Lower	Median	Upper
Propyl bromide	6.1	9.3	17.0
Butyl bromide	5.3	8.3	9.6
Ethyl iodide	4.2	5.6	8.8
Butyl iodide	3.1	4.2	5.7
Methyl thiocyanate	2.8	6.1	13.3
Ethyl thiocyanate	2.4	7.0	8.9
Propyl thiocyanate	3.3	6.8	12.0

extreme were presumed chemical toxicants, acting at very low, or low, p_i/p_s values, where loss of movement was never complete during the 5 hr. exposure time, even at concentrations which were known to cause 100% mortality. The majority of the organisms began to die a day or more after fumigation, and in the case of the iodides the kill might not have reached its final level after 4 days.

Between the extreme cases of high anaesthesia and negligible inhibition of movement, a gradation of symptoms was observed. The higher chlorides of a series, which acted at high p_i/p_s levels, were not so effective as anaesthetics as the lower ones. On the other hand, those bromides which acted at very low p_i/p_s levels were rather less effective in inhibiting movement than those of higher molecular weight. The higher iodides, acting in the range $p_i/p_s = 0.1-1.0$ for 50% kill, retarded movement to some extent during the period of exposure, but complete loss of movement was not observed at the experimental concentrations. Thus, as far as a distinction is possible, the apparent narcotic effectiveness of the compounds employed was in the order

chloroform = lower chlorides > hydrocarbons > upper chlorides
= alcohols = middle bromides > thiocyanates \approx iodides and lower bromides.

DISCUSSION

Concentration causing 50% kill

Relation between the logarithmic molar median response concentration and the number of carbon atoms in the molecule

An inspection of the lines in Fig. 1 indicates three things. In the chloride and hydrocarbon series, the points are, within experimental error, arranged linearly and the lines are of high slope, corresponding to large decreases in the external molar LD 50's as the series are ascended. For such series there can be little doubt that the action of all the compounds is primarily physical, depending only on the molar concentration and not upon the nature of the narcotic which dissolves in the (lipoprotein) biophase. At the other extreme are the lower members of the iodide, bromide and thiocyanate series, the toxicities of which are not related in any obvious manner, and which, *a priori*, might be expected to have specific chemical actions. Between the two lie the interesting series of higher bromides, iodides and thiocyanates, for which the logarithmic molar LD 50's decrease regularly within each series, but by an amount far less than those of the physically active series. The progression

$$1 : 1/Z : 1/Z^2$$

represents the relationship between the molar toxic concentrations in a series whenever a regular decrease in the logarithmic toxic concentrations is evident. For the physically acting series, the constant divisor, Z , is typically between 2 and 3, whereas for such series as the iodides it is only a fraction greater than unity. Compounds of the higher iodide type have here been termed physico-chemical poisons,

but it is not intended that these should be regarded as a third group distinct from both the chemical and the physical poisons. The term has also been used by Ferguson & Pirie (1948), but in a somewhat different way.

The alcohols, which are normally regarded as typical narcotic substances, are anomalous in that the slope of the line is of the same order as that of the 'physico-chemically acting' iodides and thiocyanates, and the question arises whether the same factors are responsible for the departure from the Traube-Richardson rule (where $Z=2$ to 3) in all three cases. The slope of the alcohol line depends very largely on the organism employed and upon the method of administration, so that

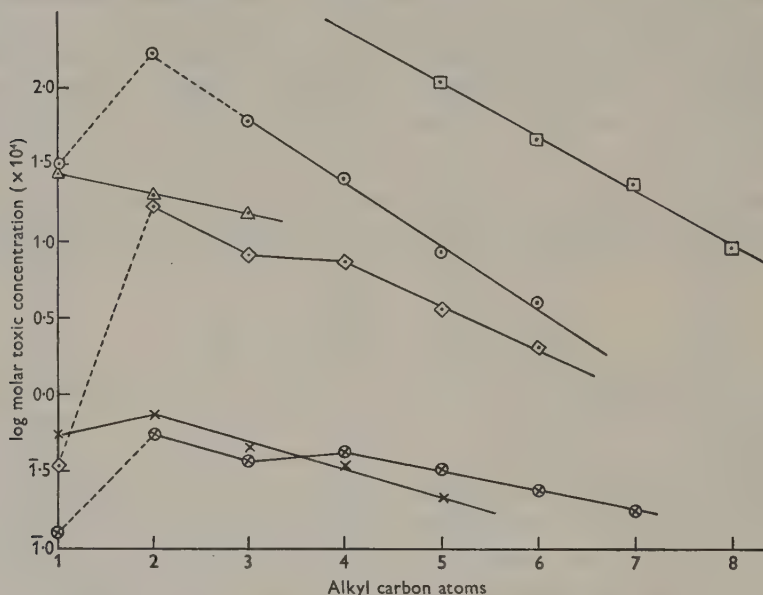


Fig. 1. Relationship between logarithm of median response concentration and number of alkyl carbon atoms. □ Hydrocarbons. △ Alcohols. ⊗ Iodides. ○ Chlorides. ◇ Bromides. × Thiocyanates. The dotted lines show three points determined by Ferguson and Pirie (1948).

the apparent similarity noted above with the physico-chemically acting series may be due to quite different factors, such, for example, as association. On the other hand, the physico-chemical poisons may also act differently when applied in other ways and more data upon the toxicities of aqueous solutions of bromides and iodides seem very desirable.

The results of Ferguson & Pirie (1948) show an alcohol regression line of much higher slope, comparable to that of bromides, from which it appears that even with different populations of one species of organism, the relative response to different homologues can vary quite considerably. Again, alcohols applied as aqueous solutions to various organisms, isolated tissues and cell constituents often show the

typical physical effect, such that the relative saturation p_i/p_s only increases slowly as the series is ascended, and cases have been reported where this value even appears to be constant or to decrease slightly. As examples of the first types of action may be mentioned the inhibition of the activity of *Paramecia* (Kamm, 1921), the narcosis of tadpoles (Overton, 1901) and the precipitation of nucleoprotein (Batelli & Stern, 1913), while Brink & Posternak (1948) discuss toxicity data, both obtained by themselves and quoted from Meyer & Hemmi (1935) where the gradual increase in p_i/p_s did not occur. The toxicity data of Vernon (1913), using aqueous solutions of alcohols, with tadpoles as the test organisms, are significant in that the ratios of the external toxic concentrations, and hence the relative magnitudes of the p_i/p_s values, were different according to the ages of the organisms.

Comparison of results

A comparison of the results of this study with those obtained by Ferguson & Pirie (1948), using the same species of organism and rather similar experimental conditions, shows a broad measure of agreement both with regard to the absolute molar concentrations (and thus the p_i/p_s values) of each substance necessary to kill 50 % of the organisms in 5 hr., and in the relative toxicities of the substances. Three of Ferguson & Pirie's results have in fact been added to complete Fig. 1. The following points are of interest, however.

First, as mentioned above, the line for the alcohols is much flatter than that obtained by the previous workers. Secondly, the p_i/p_s values obtained by Ferguson & Pirie rise more slowly in most homologous series than those recorded here—i.e. the ratios of the molar external toxic concentrations approached in their case more nearly the ratio

$$1 : 1/3 : 1/3^2$$

characteristic of many physical properties of substances in homologous series. Thirdly, and possibly related to the previous observations, the points of commencement of the straight lines in Fig. 1 were occasionally slightly different in this work from those found by Ferguson & Pirie, the commencement of linearity being one member earlier in both the bromide and iodide series in their case. It may be that some of these differences reflect a difference in the sensitivity of the site of action to a given poison in the two beetle populations. The possibility also exists, however, that the cuticle or other 'intermediary biophase' (Hurst, 1943) is slightly different in the two cases, presenting rather more of a barrier in one case than the other. If narcotic action does depend largely on molar concentration at the biophase, differences in the absolute magnitudes of the LD 50's for a physical poison in the two cases could well be due to biophases of different sensitivity, but the differences in relative magnitude of toxic concentrations of a series of such compounds in the two cases could also be explained in terms of penetration differences.

The relative saturation, p_t/p_s , in relation to the type of action

It was stated above that the term 'physico-chemical toxicity' was used to describe those compounds for which a logarithmic decrease in external toxic concentration occurred as the series was ascended, but for which the decrease was less than that typical of physical action. Ferguson (1939) has shown that for many chemically acting compounds the relative saturation is very low, and that for compounds often regarded as physically toxic it is usually within the range of $p_t/p_s = 0.1-1.0$. Ferguson & Pirie (1948) determined the lethal doses of a large range of substances against *Calandra* and divided them into physical, chemical and physico-chemical poisons, according to the magnitudes of the relative saturations, or 'activities' at which they operated. For the physical compounds, an equilibrium is considered to be established between drug and biophase, so that for these, the relative saturation becomes a fair measure of the thermodynamic activity. For chemically acting substances, the relative saturation may not have this significance, since an equilibrium is often not achieved. For the physico-chemical ones, often acting in the relative saturation range of 0.05-0.3, it is not known whether an equilibrium is established or not. This rather arbitrary division leads to a few difficulties, particularly if the limits of relative saturation defining physico-chemical action are presumed to be the same for all groups of substances. For example, chloroform (relative saturation 0.20), ethyl chloride (0.28) and acetone (0.15) are classified as physically active, whereas amyl bromide (0.32) is considered to belong to the physico-chemical poisons.

In the present study, toxicities of alkyl bromides, iodides and thiocyanates were investigated up to the highest effective member. On plotting log molar LD 50's against the number of alkyl carbon atoms, the difference between the log concentrations for successive homologues becomes constant after a certain point in each series. The slopes of the straight lines in Fig. 1 reflect the magnitudes of these logarithmic differences. Where such a constant factor relates succeeding LD 50's in a series, it seems likely that the mechanisms of action of the compounds concerned have much in common, and in particular are more alike than those of substances whose concentrations are not so related. If therefore butyl iodide is regarded as a physico-chemical poison, it seems desirable to term heptyl iodide a physico-chemical toxicant too, notwithstanding the high value of the relative saturation at which it operates. In this way a classification of types of action for one method of drug administration is based upon the way LD 50's (or the corresponding p_t/p_s values) change from one member to the next in homologous series, rather than upon the absolute magnitude of the p_t/p_s ratio. Thus the non-linear members of the series (Fig. 1) may be regarded as chemical toxicants, the linear ones being physically active if the slope is high and physico-chemical if the slope is lower. Whether a qualitative difference exists between physico-chemical action and the other two types is however uncertain. It may be an intermediate type merging into both of the others, or a special aspect of one of them.

It is worthy of note that the linearity commences at different values of the relative saturation in the different series, the lowest being 0.06 for the iodides, a value in fair agreement with the one of 0.05 suggested by Gavaudan, Dodé & Poussel (1944) and Gavaudan (1947) as the lowest possible value for a *physically* toxic compound. According to their theory, this value must apply for all compounds completely miscible with the biophase. As the relative saturation usually rises in a series, the higher homologues must be considered to be less soluble in the biophase than the lower ones, assuming as Gavaudan does, that equal numbers of molecules of such substances in the biophase have equal narcotic effects. However, since their mathematical treatment was based upon experimental work quoted by Meyer & Hemmi (1935) in which olive oil was used as the model biophase, and for which this solubility relation does not hold, the value of 0.05 is somewhat speculative, especially since the observed relative saturation at which a narcotic operates also depends upon time of exposure.

Types of toxic action

At least two types of biophase exist, which differ with regard to the phase-distribution relationships between them and the external medium. There is a lipid or lipoprotein biophase in which the physical poisons are believed to function, and for which the biophase/external medium distribution coefficient increases approximately threefold from one member of a homologous series to the next higher one. In addition, there is an aqueous biophase in which many ionic chemical poisons operate. Here, as in an external vapour or aqueous phase, the absolute solubility of organic compounds rapidly decreases as a series is ascended.

There is evidence that the relative toxicities of physical poisons may be the same, or nearly so, when tested against different types of organisms (Busvine, 1942), and McGowan (1951, 1952) has calculated series of additive factors whereby the toxicities of such compounds may be predicted with fair accuracy in many cases. The action of physical poisons is primarily reversible, usually occurs at relative saturation values of between 0.05 and 1.0, and although their precise mechanism of action is not known, equimolar quantities of all appear to bring about nearly the same response when applied under similar conditions.

In addition to this physical action, which does not depend upon chemical interaction of drug and cell component, it is theoretically possible for a toxic substance to react with some constituent of either an aqueous phase or lipid phase. Assuming a vital system vulnerable to the toxicant to be present in either or both phases, the observed toxic effect for a given exposure time will depend on the speed of interaction as well as on the concentration of drug. Thus if two homologues have the same chemical action on a certain biophase component, but one is twice as reactive as the other, less of the more reactive one would be necessary to produce a given effect in unit time. This would be reflected in the magnitude of the respective external toxic concentrations after allowance had been made for the appropriate

phase distributions. For two such compounds, a distinction must be made between *mode* of action, identical in the two cases, and the *intensity* of action, which could be very different.

Three other complications exist. First, compounds possessing suitable solubilities, as well as distributions and reactivities, could react simultaneously in two phases. Secondly, although chemically acting substances often operate at low p_i/p_s values, there seems to be no reason why this need be so. Substances of moderate chemical reactivity could act at high p_i/p_s ranges, especially if the time of exposure were short. Such substances might then be expected to have a combined chemical and physical effect. Thirdly, as mentioned earlier, barriers such as the insect cuticle could affect the speed of entry, or more likely of exit, of certain substances.

Very little is known about the chemical reactions of simple alkyl derivatives in living organisms, and therefore an analogy must be made between toxic action and general chemical reactivity *in vitro*. This is made easier, and partly justified, by the fact that the behaviour of reaction velocity constants within a series is mainly independent of the type of reaction, at least for the substitution and complex formation reactions so far investigated. Within the three alkyl halide series, moreover, chemically similar reactions have been studied under identical conditions, and have led to a knowledge of relative reaction rates across as well as within, series. Experiments have shown that: (a) chemical reactivity increases in the order chloride, bromide, iodide; (b) within each series, the reactivity is greatest for the methyl compound and decreases rapidly to a nearly constant value characteristic of propyl and higher members.

Fig. 1 shows that, at least for the halides, the slopes of the straight lines relating median response dosage to the number of carbon atoms is greatest where the *in vitro* reactivity is very low. This supports the view that the toxicity of compounds following the Traube-Richardson rule (for which $Z=2$ to 3) is not to be ascribed primarily to chemical reactivity, but that the latter could account for the lower slopes of the bromides and iodides. Also, the linear relation between the number of carbon atoms and median response dosage in Fig. 1 breaks down at or near a point predictable from *in vitro* reaction rates, on the assumption that if two compounds undergo the same reaction *in vivo*, their toxicities will be related to their rates of reaction with the substrate.

Shapes and slopes of probit lines

Shapes of probit lines

For about three-quarters of the twenty-five substances for which regression lines were obtained, toxicity tests gave series of points through which straight lines could be drawn, while for the remainder, upon fitting the best straight line, there was some evidence of curvature. All the compounds accepted as physical poisons, including the alcohols which behaved anomalously in not obeying the Traube-Richardson rule, gave linear graphs, but for the remaining series the connexion between type of activity and type of probit graph was not apparent. In several cases,

for example, substances believed to be chemical toxicants gave straight lines, while higher members appeared to give curved graphs. There does not therefore appear to be any reason for associating curved lines with chemical toxicity, especially as it is well known that many highly toxic materials give straight regression lines. So far as any generalization is permissible, it would appear that probit lines are most curved before or at the points in Fig. 1, where linearity commences, and, upon further ascending the series, tend to become linear by the time the homologues next to the cut-off members are reached.

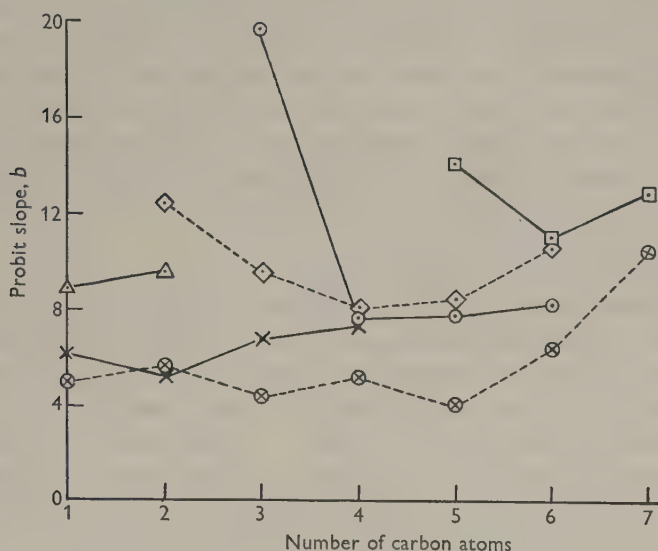


Fig. 2. Relationship of probit slope to number of alkyl carbon atoms. □ Hydrocarbons. △ Alcohols. ⊗ Iodides. ○ Chlorides. ◇ Bromides. × Thiocyanates.

Several investigators, for example Tattersfield & Martin (1935) and Proverbs & Morrison (1947), have noted that 'breaks' occasionally occur in probit regression lines, the points corresponding to low concentrations being on a lower slope than the rest. Finney (1952, p. 68) also mentioned this effect. In most cases, however, the points have been fitted quite satisfactorily by two intersecting straight lines, rather than by a continuous curve. This may possibly be due to the fact that in nearly every case there have been less than 8 points on each probit graph, whereas in the present work (Table 3) there were generally between 20 and 30 derived from two or more experiments.

For the seven substances for which curvature was most apparent, upper, median and lower probit lines were calculated, and the results in Table 4 indicate the degree of departure from the general regression line. Since, however, more extreme dosages were used for some substances than for others, comparison of the higher (or lower) slopes amongst themselves could be misleading.

Slopes of probit lines

The slopes of the probit regression lines, together with their variances and the number of points on each graph, appear in Table 3.

Excluding the hydrocarbons and alcohols, for which the data are in some ways unsatisfactory, there appears to be a tendency for the probit slopes at first to fall as a series is ascended and then to rise again. High toxic vapour pressure is often associated with a high relative change of slope. For the hydrocarbons and alcohols the slopes within each series are not significantly different, but for the latter the small rise may nevertheless be real, since more recent work carried out two years after that reported here shows a consistently higher slope for ethyl alcohol than for methyl. This is probably analogous to the rise near the cut-off point noted in other series. It is of particular interest that the slopes fall and rise fairly regularly, even though at $P=0.05$ the differences between these slopes for two consecutive homologues are not always significant. The probit slope of propyl iodide (Fig. 2) is an exception to this regularity, and this compound, unlike its neighbours, appeared to give a straight probit line.

There is probably no connexion between the fall and rise of the slopes of probit lines within a series of compounds, and the types of activity shown by the toxicants, despite the fact that the minimum slopes of probit lines sometimes occur at or near the points in Fig. 1 where the straight lines commence. The most striking change of slope is evident in the cases of the chlorides for which no change of type of toxic action is suspected. Moreover, the change in probit slopes of the physical and physico-chemical poisons as the series are ascended shows that toxicants with similar modes of action do not always give probit lines which have the same slope. The very different slopes of the lines of chloroform, alkyl chlorides and hydrocarbons are also interesting in this connexion, since all of these have a more or less pronounced anaesthetic effect, and are probably acting in the organisms in a similar way.

Considering now the slopes of the central members of the series, which do not differ greatly within each group from one another, it is found that the compounds can be divided into two groups, for the first of which the slopes are in the range of 8 to 15, for the second between 4 and 7. The iodides and thiocyanates, which comprise the latter group, differ from the remaining substances in Fig. 1 in acting at very low external toxic concentrations or vapour pressures. There may therefore be a direct connexion between slope of probit line and the magnitude of the toxic vapour pressure (Fig. 3), or an indirect one between the slope and chemical reactivity, when a comparison is made across series. In view of the differing slopes for physically toxic compounds and also the high slope for ethyl bromide, which is believed to be chemically toxic, the first seems more probable than the second. However, this correlation could not fully explain the results obtained for slopes within series, and there is evidence that the problem will prove complex.

Busvine (1938) found that when ethylene oxide was used as a fumigant for exposure times varying from $\frac{1}{2}$ to 20 hr., the probit slopes at the different times varied very considerably. Moreover, they behaved differently for each of the four types of organism tested, even though the toxic concentrations were of the same order for each exposure time. In the case of *C. granaria*, the slope rose to a maximum of 14 at 2 hr. and then fell to 7 after 20 hr., but for the other three types of organism that he used no such regularity was evident. Work which will form the subject of a later paper, and supplements the results in Tables 2 and 3, has confirmed Busvine's findings, showing that when several substances are applied as fumigants to one test organism for different periods of time, the slopes of the probit lines change, some-

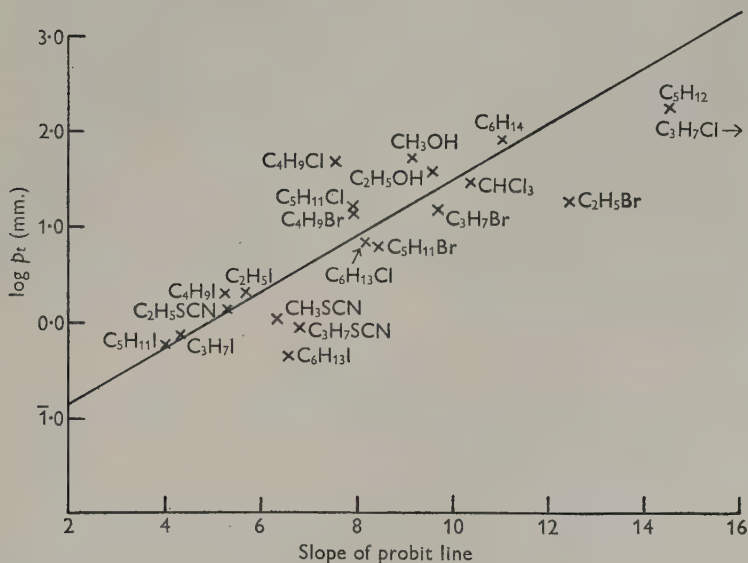


Fig. 3. Relationship of probit slope to $\log p_i$ for substances acting below $p_i/p_s = 0.8$.

times increasing and sometimes decreasing with a longer exposure time. Lord (1948) obtained different relative slopes for several DDT compounds when these were tested against two different types of organism. Lastly, Proverbs & Morrison (1947) obtained different probit slopes according to the way in which DDT analogues were applied to the organism. Thus, excluding the possibilities that the differences of probit slope are due in some way to experimental error or method, or are in some way bound up with the double transformation probit method, it would appear that relative probit regression slopes can be influenced by the type of organism, the time of exposure and method of application, as well as by the nature of the substances, and hence a complete explanation in terms of any one chemical factor, such as vapour pressure, seems unlikely.

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THE CONTROL OF BLOWFLIES INFESTING SLAUGHTER-HOUSES

II. LARGE-SCALE EXPERIMENTS

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Field experiments on the control of blowflies have been carried out at a large slaughter-house which was heavily infested both by flies breeding on the premises and by those attracted from surrounding districts.

The control of breeding by admixture of a larvicide with slaughter-house refuse proved to be impracticable. Although a single treatment of the surface of refuse was ineffective, daily applications of DDT or BHC markedly reduced the degree of infestation and killed some emerging adults.

The introduction of a scheme for rotational storage and collection of refuse and a general improvement in sanitary conditions, together with a daily application of 5% DDT dust to refuse and nearby vegetation, resulted in the prevention of breeding on the premises and a very considerable reduction in the adult population.

Results of experimental applications are compared with those obtained in practice using unskilled labour.

INTRODUCTION

A study by Green (1951) of the habits and life histories of blowflies infesting slaughter-houses suggested various methods of controlling these insects. Some of the recommendations then made have now been given extensive trial at a large, heavily infested abattoir situated in a densely populated, built-up area. The premises cover about 8 acres and consist of eighteen slaughtering bays, each with its own hanging room, seven large chilling rooms and various other buildings connected with the by-product industries. At the rear of the premises is an open concrete yard containing refuse which consists of a mixture of congealed blood removed from traps in the drainage system, gut contents, sweepings from the slaughtering bays, pig hair and waste pieces of wool and hide. Immediately behind this midden is about an acre of waste ground overgrown with grass and weeds. The lairages, which are about 200 yd. away, cover a further 12 acres approximately.

The blowflies were the mixed population already described (Green, 1951), and consisted of *Calliphora erythrocephala* (Meig.), *C. vomitoria* (L.), *Lucilia caesar* (L.), *L. illustris* (Meig.), *L. sericata* (Meig.) and *Phormia terrae-novae* (R.-D.). They comprised those that had immigrated from nearby breeding places and those that had bred on the premises. When not in the slaughter-house buildings or yards, the flies congregated on the vegetation covering the waste ground. There was, therefore, ample scope for control of blowflies at all stages, both by improved hygiene and the use of insecticides.

PRELIMINARY EXPERIMENTS

Although, with good hygiene and frequent collections of waste materials, blowflies will be prevented from completing their life cycle at the slaughter-house, much of the refuse leaving the premises will inevitably be infested and will subsequently contribute to the general blowfly population. Various small-scale experiments have been carried out to find methods of confining such infestations to a low level.

Mixture of insecticide with refuse

McDuffie, Lindquist & Madden (1946) found orthodichlorobenzene (ODCB) very effective against larvae of houseflies and blowflies, and our laboratory experiments showed that a mixture, by volume, of 10% ODCB, 50% carbon tetrachloride, and 40% kerosene was toxic to larvae of *Lucilia sericata*. Although this mixture could probably not be used in practice, it was suitable for determining the practicability of control by admixture of a larvicide and wet refuse.

Field tests showed that the liquid was effective only when in contact with the insects, and that it could not be distributed in the refuse sufficiently evenly to obtain a good kill of larvae. Treatment with the ODCB mixture prevented neither oviposition nor development of large numbers of larvae.

A dust containing 4% crude benzene hexachloride (BHC) was ineffective against larvae when mixed with infested refuse at the rate of 1 lb./cwt. (45 p.p.m. γ -BHC).

Treatment of the surface of refuse

Several workers have shown that the application of insecticide to larval breeding media can prevent or considerably reduce infestations provided that treatment starts before the infestation develops. McDuffie *et al.* (1946) found DDT ineffective against larvae in infested carcasses but obtained good control when a carcass was sprayed before it became infested; BHC was better than DDT against established infestations and was good also as a preventive. Travis & Bohart (1946) found that insecticidal treatment of latrine pits was successful only if begun as soon as the pit was installed.

In a series of experiments carried out to determine the effect, on the subsequent degree of infestation, of various insecticides applied to the surface of fresh refuse the following insecticides were used:

- (1) *DDT dust*. 5% DDT in china clay.
- (2) *DDT spray*. 5% (w/v) DDT in Pool burning oil (P.b.o.).
- (3) *DDT emulsion*. A 20% (w/v) DDT emulsion.
- (4) *DDT/pyrethrum spray*. 0.3% (w/v) DDT + 0.03% (w/v) pyrethrins in Shell oil P31.
- (5) *BHC dust*. 4% crude BHC in gypsum.
- (6) *BHC spray*. 0.35% γ -BHC in light oil.
- (7) *Pyrethrum spray*. 0.1% (w/v) pyrethrins in P.b.o.

(8) *Shell oil P₃₁*. Oil alone used for comparison with treatment (4).

(9) *Pool burning oil*. Oil alone used for comparison with treatment (7).

For convenience and brevity, subsequent references to any of the above treatments will generally be by title only, e.g. P.b.o. (Pool burning oil), DDT dust, pyrethrum spray, etc.

Small heaps of refuse, each consisting of a mixture of blood, hair, dung, and sweepings from the slaughtering bays, were arranged along the foot of a wall running from north to south in the midden yard. Each heap had a surface area of about 5 sq. ft., weighed about $\frac{3}{4}$ cwt. when first prepared, and usually dried out to about $\frac{1}{2}$ cwt. The following methods of application were used:

Dusts. DDT and BHC dusts were shaken through 20-mesh (28 i.s.w.g.) and 30-mesh (31 i.s.w.g.) sieves, respectively.

Sprays. The kerosene sprays were applied with a pneumatic hand-sprayer, of 3 pints (1700 ml.) capacity, fitted with a lime-washing nozzle.

In the first experiment only a single application was given, but subsequent experiments were concerned with the effect of regular daily treatments.

A Howard rain gauge and a Stevenson screen containing a recording thermo-hygrograph were set up on the waste ground behind the midden. All rainfall and temperature records were taken for 24 hr. periods beginning at 10 a.m.

Effect of a single, surface treatment

Nine heaps of refuse, numbered from north to south, were treated as follows: (1) BHC dust; (2) P.b.o.; (3) DDT spray; (4) untreated heap; (5) BHC spray; (6) DDT dust; (7) P₃₁; (8) pyrethrum spray; (9) DDT/pyrethrum spray. The sprays were applied at the rate of 200 ml. per heap and the dust at 114 g. (4 oz.) per heap.

During the 4 days in which the heaps were exposed, rain (0.37 in.) fell only on the second night. Minimum night temperatures were from 49 to 50° F., and maximum day temperatures from 67 to 78° F.; the days were mostly sunny.

Within 2 days all heaps were infested with eggs and young larvae and, after 4 days, were heavily infested with all instars of larvae. Although there may have been small differences in the degree of infestation between the various heaps, it was obvious that no treatment had been sufficiently effective to warrant further work.

Effect of daily, surface treatments

Travis & Bohart (1946) found under field conditions that daily treatment with small quantities of insecticide gave the best control of flies breeding in army latrines. Heaps of refuse therefore were set up as before but were treated each day; daily rates of application were: dusts, 50 g.; oil sprays, 50 ml.; emulsion, 150 ml. of a 1:11 mixture with water (giving 2.5 g. DDT per heap, as with DDT dust). A preliminary experiment indicated that the infestation of refuse was considerably

reduced by daily treatment with some dusts and sprays, and demonstrated the need for quantitative measurement of the infestation if the differences in degree of control were to be appreciated.

A technique was evolved which entailed trapping all adults that emerged from the experimental heaps, thus measuring overall control rather than control at any one stage. The heaps of refuse were treated as before but, at the end of the exposure period of 3 days they were loaded into special containers and transported to the Laboratory. The containers were heavy-gauge, galvanized, iron bins, height 24 in., diameter 18 in. at the top tapering to 15 in. at the base: each bin had a heavy, closely fitting lid 2 in. deep. In order to prevent larvae migrating from the bins, a strip of sheet metal about $1\frac{1}{2}$ in. wide was fixed to the inside 3 in. from the top of each bin to form a ledge inclined downwards at an angle of about 45° . A hole, $2\frac{1}{2}$ in. across, was cut in the centre of the lid and a metal cylinder, 3 in. long, with a wire gauze cone-escape at the top, was fitted so that its end was flush with the underside of the lid. A further hole, $\frac{1}{2}$ in. across, was drilled in each lid so that rubber tubing could be led through for delivering air into the bins from a pump. A 6 cm. filter funnel was fixed to the end of the air tube to give a wide aperture, and its mouth was covered with wire gauze to prevent the entry of migrating larvae which otherwise crawled up and blocked the tube.

Before loading, the bottom of each bin was covered with about 4 in. of peat, and the refuse added carefully so as to disturb it as little as possible and leave the treated surface at the top. At the Laboratory the bins were stored out of doors but sheltered from rain. Tubing from the aerating pump was led into each bin so that the funnels rested on the refuse and air was blown in continuously, otherwise a high concentration of ammonia was built up, particularly when there was a dense larval population. The bins were, at first, covered only with muslin because when the lids were in place there was considerable condensation inside the bins, particularly during the first few days of an experiment, and larvae were able to travel in the film of moisture and escape over the metal barrier. When fully grown larvae began to migrate from the food, a rough cone of crumpled sacking was placed on top of the refuse above the air inlet and was partly buried in additional peat: it was found that the majority of the larvae burrowed through the peat, travelling round the periphery of the bin for some time before pupating in the sacking. As soon as flies began to emerge, the lids were placed on the bins and, to ensure darkness inside, paper was stuffed between the bin and the overlapping flange of the lid. A stockinette sleeve on a cylindrical, wire frame, 9 in. high and 6 in. across, was secured on the top of the lid so that emerging flies, attracted to the only source of light, passed through the cone-escape and were trapped in the sleeve. Each day the sleeves were changed and the trapped flies were killed, classified (*Musca* spp., *Calliphora* spp., *Phormia* spp., *Lucilia* spp.), counted and sexed.

For each experiment, six heaps of refuse were set up and treatments applied as in Table 1. There was intended to be only one pair of duplicated experiments but,

although the number of replicates was increased to four, ambient conditions were such that no two were carried out under similar conditions of weather and insect population. There was comparatively little oviposition during Exps. 1 and 3 which were, therefore, repeated without changing the order of treatment.

A summary of the weather conditions during the treatment period is given in Table 2.

During the exposure period in Exp. 1 there was little sunshine and the temperature was above 65° F. for only 4 hr. This reduced the activity of the flies so much that no treated heap became infested, and the untreated heap contained very few larvae; the experiment was, therefore, discontinued and the heaps discarded. In the repeat experiment (no. 2) the weather conditions were more favourable for oviposition, particularly during the first 2 days. By 4 August 1948, when Exp. 3 was started, the infestation by blowflies was greater than on any previous occasion during the year, owing to a great increase in the numbers of *Lucilia*; the *Phormia* population was declining. Despite the increase in numbers of adults, the infestation

TABLE 1. *Treatments and arrangement of refuse heaps for daily surface applications of insecticide*

Treatment	Heap number	
	Exps. 1 and 2	Exps. 3 and 4
DDT dust	5	2
DDT emulsion	3	6
DDT/pyrethrum spray	2	3
BHC dust	6	4
Pyrethrum spray	1	5
Untreated	4	1

of the experimental heaps was comparatively light because of inactivity of the flies during sunless weather. The weather did not improve sufficiently for the experiment to be repeated until the end of August. The adult population was then increasing very rapidly as a result of the infestation, chiefly by *Lucilia*, of about 1 ton of refuse which was left in the yard during the very hot weather over August Bank Holiday and from which a very great number of larvae migrated before it was collected. On 31 August 1948 the repeat experiment (no. 4) was carried out, and for the first half of the exposure period conditions were more favourable for oviposition: the great majority of the eggs were probably laid on the second day of exposure. The results of the four experiments are dealt with under a series of subject headings.

The effect of the treatments on the numbers of flies visiting the heaps

Thirty minutes before and after each application a count was made of the number of flies which were in contact with each heap at any time during a 1 min. observation period. A complete set of counts was obtained only in Exp. 2, and these are

TABLE 2. *Daily surface treatment of refuse: a summary of weather conditions during the periods of exposure for oviposition*

Experiment no.	Date started	Rainfall (in.)	Temp. (°F.)		General observations
			Max.	Min.	
1st day					
1	14. vii. 48	0	70	55	Sunny periods
2	19. vii. 48	0	82	62	Warm and sunny all day
3	4. viii. 48	0	71	56	Warm but over-cast all day
4	31. viii. 48	0.06	79	54	Warm and sunny all day; rain at night
2nd day					
1	14. vii. 48	0	61	53	Cloudy; no sun
2	19. vii. 48	0	79	62	Sunny all day but very windy
3	4. viii. 48	0.11	65	60	Overcast all day; rain evening and night
4	31. viii. 48	0	71	54	Cloudy; sunny periods
3rd day					
1	14. vii. 48	0	67	48	Cloudy; a few short sunny periods
2	19. vii. 48	0	74	52	Cloudy and windy all day
3	4. viii. 48	0.61	68	61	Intermittent rain all day; treatments applied during heavy rain
4	31. viii. 48	0.19	67	56	Dull, cloudy; steady drizzle all day
4th day					
1	14. vii. 48	0.23	65	57	Short, sunny periods; rain at night
2	19. vii. 48	0	74	50	Sunny all day but windy
3	4. viii. 48	0	74	57	Sunny periods
4	31. viii. 48	0.06	67	54	Cloudy with short, sunny periods. Heaps loaded into bins at 3 p.m. during heavy rain

summarized in Table 3. It was shown that, by comparison with the untreated heap, all treatments reduced the number of flies visiting the heaps; the sprays containing pyrethrum were more repellent than the other treatments. The increased numbers 'after treatment' on the control heap were almost certainly due to repellency of the treated refuse.

The effects of the treatments on larval infestation

In Exps. 2-4 the numbers of flies placed in the classes referred to previously have been totalled for each treatment. The results are given in Table 4; the total emergence figures include the flies which entered the trap sleeves and those which died in the bins.

All treatments achieved some measure of control, but the degree was related to weather conditions. In cool, rainy weather there was limited oviposition on untreated material alone, whereas in warm, sunny weather both treated and untreated materials became heavily infested; although flies showed a marked preference for the untreated heaps at all times.

TABLE 3. *Daily surface treatment of refuse: the repellent effect on adult blowflies*

Treatment (Exp. 2)	No. of flies settling in 1 min.							
	19. vii. 48	20. vii. 48		21. vii. 48		22. vii. 48	Total	
		Before treat- ment	After treat- ment	Before treat- ment	After treat- ment		Before treat- ment	After treat- ment
DDT dust	0	15	6	4	0	9	28	6
DDT emulsion	2	20	20	3	5	5	28	27
DDT/pyrethrum spray	0	10	0	4	0	12	26	0
BHC dust	1	20	12	3	1	17	40	14
Pyrethrum spray	0	15	0	4	0	17	36	0
Untreated	14	50	100	4	8	38	92	122

DDT treatments were the most effective, especially in Exps. 3 and 4 where the infestation was predominantly *Lucilia*. As applied in this work, DDT is apparently more effective against *Lucilia* than against *Phormia*. In addition to reducing the degree of infestation, three treatments, DDT emulsion, DDT dust, and BHC dust, killed an appreciable number of emerging flies which died in the bins. Furthermore, with these treatments, about 50% of those flies which passed through the cone-escapes and were collected in the sleeves were affected in varying degrees by the insecticides. Olson & Dahms (1945) and Furman (1946), using DDT for the treatment of sewage sludge and latrine pits respectively, showed that the majority of emerging flies died within a short time of emergence.

In Exp. 2, the infestation in the untreated heap consisted almost entirely of *Phormia* and bore little relationship to the natural population of the slaughter-house (which consisted of probably not more than 40% *Phormia*) or to the species distribu-

tion in the treated heaps. It has been observed that *Phormia* tend to oviposit on fresher refuse than *Lucilia* and to be less attracted to stale, rapidly decomposing material; furthermore, as shown by Green (1951), their larvae develop more rapidly than those of *Lucilia*, and are predaceous. Under these circumstances, the initial infestation would be by *Phormia* and, because of the repellency demonstrated in Table 3, would be much heavier in the untreated heaps. Subsequent infestation by *Lucilia* would succeed only in the treated heaps where competition from *Phormia*

TABLE 4. *The effect of the daily, surface treatment of refuse on the numbers of blowflies subsequently emerging*

		No. of flies											
Exp. no.	Classification of flies	DDT dust		DDT emulsion		DDT/pyrethrum spray		BHC dust		Pyrethrum spray		Untreated	
		Total no. emerging	No. dead in bin	Total no. emerging	No. dead in bin	Total no. emerging	No. dead in bin	Total no. emerging	No. dead in bin	Total no. emerging	No. dead in bin	Total no. emerging	No. dead in bin
2	<i>Musca</i>	16	5	33	9	120	14	57	5	214	0	0	0
	<i>Calliphora</i>	3	3	6	0	3	0	0	0	117	4	3	0
	<i>Phormia</i>	7,493	2,228	1,800	1,016	1,701	31	4,660	35	957	4	14,695	4
	<i>Lucilia</i>	819	461	740	243	6,017	115	3,276	778	8,623	408	102	0
	Total	8,331	2,697	2,579	1,268	7,841	160	7,993	818	9,911	416	14,800	4
3	<i>Musca</i>	0	0	0	0	0	0	0	0	0	0	0	0
	<i>Calliphora</i>	0	0	9	0	9	2	346	250	5	5	2,139	0
	<i>Phormia</i>	18	9	11	4	32	0	189	57	0	0	330	0
	<i>Lucilia</i>	9	7	620	16	179	3	694	333	2	0	3,902	1
	Total	27	16	640	20	220	5	1,229	640	7	0	6,371	2
4	<i>Musca</i>	25	0	0	0	0	0	77	0	0	0	954	0
	<i>Calliphora</i>	3	1	4	2	7	2	164	99	10	0	1,787	0
	<i>Phormia</i>	50	22	0	0	0	0	83	4	0	0	612	0
	<i>Lucilia</i>	288	88	122	24	349	29	5,061	2,704	1,267	48	22,514	1
	Total	366	111	126	26	356	31	5,385	2,807	1,277	48	25,867	1
Grand total (3 experiments)		8,724	2,824	3,345	1,314	8,417	196	14,607	4,265	11,195	464	47,038	8
Percentage of total emergence dying in bins		32.4		39.3		2.3		29.2		4.1		1.8	

was less fierce. By the time that Exps. 3 and 4 were carried out *Phormia* represented somewhat less than 5 % of the total adult population and were unable to influence results in the same way.

The most promising treatments, as judged by reduction in larval infestation and kill of adult flies, were DDT emulsion and DDT dust. The dust was adopted for regular use because its practical advantages outweighed the small loss in effectiveness. BHC dust, although giving a good kill of adult flies, was less effective than DDT in reducing larval infestation.

Tests for repellency of DDT

At night and during cool, cloudy weather, a very high proportion of the infestation of blowflies rest on the vegetation at the rear of the premises. Before undertaking any large-scale treatment of this vegetation, it was necessary to ensure that there

was little risk of repelling flies from their normal resting places and driving them to areas which would be difficult to locate and treat or into the yards and slaughter-houses. Accordingly, two plots, each of about 600 sq.ft. were treated with an aqueous dispersion of DDT wettable powder and a 5 % DDT dust respectively. The spray and dust were applied at about 375 and 200 mg. DDT per sq.ft. of ground area respectively, these being judged visually, according to the deposits on the vegetation, to be the maximum doses likely to be given in practice.

There were no obvious differences between the numbers of flies resting in corresponding places in the treated and untreated areas during the 2 days following treatment. It was therefore concluded that either method could be used without risk of repelling the flies.

FULL-SCALE EXPERIMENTS

Towards the end of August 1948, the population of flies on the slaughter-house premises was higher than at any previous time that year and appeared to be increasing. It was therefore decided to carry out a full-scale trial, putting into operation as many as possible of the methods previously discussed (Green, 1951).

Hygiene

Arrangements were made for blood and other by-products to be cleared more frequently, when possible every 2 days, and for destructible waste to be burned daily. The system of rotational storage and collection previously described by Green (1951) was adopted so that refuse normally remained in the midden yard for no more than 2 days.

As a result of these measures, which were continued for three summers, infestations of larvae were virtually eliminated on premises which had previously suffered extremely heavy infestations.

Chemical control

In previous experiments, DDT was the most effective insecticide used for the surface treatment of refuse and, for large-scale use, the 5 % dust had several practical advantages: it was relatively cheap, could be used both on vegetation and in the yards, needed no preparing, and could be applied by means of simple apparatus and unskilled labour.

The first application was made on 7 September 1948 when the vegetation on the waste ground behind the midden, the surface of all refuse, and the midden yard were heavily dusted by means of a commercial bellows-type powder blower with a capacity of about 1800 g. (4 lb.). For the next 6 weeks a daily dusting was made of all refuse, parts of the yards seen to be attracting flies, heaps of horn, bins of inedible offal, and a strip of vegetation, 10 ft. wide, immediately behind the midden wall. During the subsequent two summers, this daily practice was continued and all vegetation within 50 yd. of the midden was given a heavier dusting at 6-week

intervals. The dusting was normally carried out between 3 and 4 p.m., when most of the refuse for the day had been deposited in the yard, so that all refuse was given at least one dusting before it became very attractive to ovipositing blowflies. Furthermore, at this time of the day, a high proportion of the fly population of the slaughter-house was normally concentrated on and around the midden and was killed by direct dusting.

Owing to the great variation in the density and location of local populations of blowflies caused by weather and working conditions, and to the varying situations of the most attractive materials, it was found very difficult to obtain a quantitative method of measurement of the degree of infestation. The method finally adopted was to sweep with a net over the most densely populated areas and to record daily the maximum number of flies caught on any one sweep over 2 sq.yd. of the vegetation and of the refuse. The number caught on the refuse was probably subject to greater variation because of the varying amounts of material present: if one small heap of stale refuse were present in an otherwise empty yard, almost all the flies in the vicinity would congregate on it, and a sweep with the net would give a very misleading impression of the density of the population in the yard. Furthermore, in warm weather, stale refuse seemed to attract flies from a large area beyond the slaughter-house premises.

When the first application was made the weather was cloudy and cool (65–70° F.) and large numbers of blowflies were clustered on the vegetation: a sweep with the net yielded 201 *Lucilia* and 2 *Phormia* from the vegetation and 40 *Lucilia*, 8 *Calliphora*, and 4 *Phormia* from the refuse. During the 30 min. following the application there was a haze, about 3 ft. high, above the vegetation caused by rapidly flying, affected flies. By 1 hr. after treatment not more than 100 flies could be found on the entire premises and a continuous buzz was heard from badly affected flies in the vegetation.

Results of the daily assessment of population, together with a general note on weather conditions, are summarized in Table 5.

The dusting treatment clearly caused a very marked reduction in the blowfly population and the combined effects of daily dusting, rotational storage and clearance of refuse, and improved conditions of hygiene, kept infestation of the premises at a very low level. After the heavy resident population of adults had been destroyed the density of the small residual population, most of which was considered to have flown in daily from the surrounding district, was related to weather conditions and the amount of attractive material in the yard.

A study of the effect of the control measures under fully practical conditions was made during 1949 and 1950 when responsibility for their implementation had passed to the slaughter-house authorities. Their unskilled labourers applied the dust unevenly and in doses which varied considerably from day to day. A change of operators after the 1949 season resulted in far less efficient treatment in 1950. Relatively few visits could be made to the slaughter-house but they were spaced as

evenly as possible to cover the entire blowfly breeding season. Populations were again measured by sweeping with a net over 2 sq.yd. of the most densely populated part of the vegetation. Results are summarized in Table 6, and compared with those

TABLE 5. *The combined effects of chemical control and improved hygiene on the numbers of blowflies at a slaughter-house*

Date	Max. no. flies caught per sweep		General observations
	Refuse	Vegetation	
30. viii. 48	239	184	Hot, sunny
31. viii. 48	340	250	Warm, sunny; rain at night, 0.06 in.
1. ix. 48	326	226	Cool, sunny periods
2. ix. 48	32	228	Dull, cloudy; slight drizzle; rain at night, 0.19 in.
7. ix. 48	52	203	Dull, cloudy; counts made before treatment
<i>Dust treatment started</i>			
8. ix. 48	90	61	Warm, sunny; almost all flies in one small area in the vicinity of the only refuse in the yard
9. ix. 48	65	18	Hot, sunny
10. ix. 48	108	14	Warm, sunny; only one small heap of refuse in the yard
11. ix. 48	46	10	Warm, sunny periods
13. ix. 48	18	6	Warm, sunny periods. Heavy rain, 0.79 in. on previous day (Sunday)
14. ix. 48	25	4	Warm, sunny
15. ix. 48	14	2	Warm, sunny, windy
16. ix. 48	12	1	Warm, sunny
17. ix. 48	11	2	Warm, sunny
18. ix. 48	37	2	Warm, sunny
20. ix. 48	12	0	Warm, sunny
21. ix. 48	3	0	Cool, sunny
22. ix. 48	0	0	Cool, sunny; only twenty-five flies seen on entire premises
23. ix. 48	0	0	Warm, cloudy; only fourteen flies seen
24. ix. 48	15	2	Warm, sunny
25. ix. 48	16	6	Warm, sunny; several tons of refuse, up to 3 days old, in one bay owing to breakdown in collecting arrangements
27. ix. 48	7	4	Warm, sunny; refuse now up to 5 days old but only slightly infested
28. ix. 48	31	3	Hot, sunny; refuse, 6 days old, cleared after counts were made
30. ix. 48	8	2	Warm, sunny

TABLE 6. *A comparison of the efficacy of treatments applied experimentally and by the slaughter-house staff*

Year	No. of days on which observations were made	Average no. of flies per sweep
1948 (before treatment)	5	218.2
1948 (experimental treatment)	16	2.8
1949 (unskilled labour)	16	6.9
1950 (unskilled labour)	9	15.1

obtained before and during experimental treatment in 1948. Counts for the period 8 and 10 September 1948 inclusive are not comparable and have been omitted because not all of the original resident population had been destroyed.

Results illustrate clearly the reduced degree of control obtained with unskilled labour but, nevertheless, show that under conditions of normal practice the blowfly population can be kept at a comparatively low level. There was no indication of increased resistance of blowflies to DDT during the 3 years of treatment.

Although breeding on the premises was prevented by improved hygiene, the daily immigration of adult blowflies was always sufficiently large to warrant continued chemical control. Scudder (1949) concludes that, if attractive material is exposed, even on otherwise immaculately clean premises, a large population may be built up within an hour by blowflies which have stopped there during their ranging in search of food or breeding media. Clearly the most effective way of dealing with such a problem is to control these flies at their breeding grounds rather than deal with them piecemeal after they have migrated. More recent work has therefore aimed at investigating breeding places other than slaughter-houses.

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This work has been carried out as part of the programme of research of the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research.

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FIELD STUDIES ON THE INFLUENCE OF WEATHER CONDITIONS ON EGG-LAYING BY THE CABBAGE ROOT FLY, *ERIOISCHIA BRASSICAE* BCHÉ. I

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(With 2 Text-figures)

The duration and intensity of egg-laying by the spring generation of *Erioischia brassicae* Bché has been studied by removing daily the soil around the stems of cabbage plants on an observation plot. In 1952, eggs were first found on 22 April; the peak period of egg-laying lasted from 30 April to 22 May, and in the last week of May the rate of egg-laying declined rapidly. In the peak period the rate of egg-laying was 5-46 eggs per day per plant with a mean rate of 23 eggs per day per plant. The spring infestation on observed plants was 258-817 eggs with a mean of 533 eggs per plant.

Variation in the daily rate of egg-laying was associated with weather conditions. Warm sunny weather induced heavy egg-laying but in cool, wet and windy weather fewer eggs were laid.

A summer peak of egg-laying associated with the second generation of *E. brassicae* occurred in late June and early July. Attack in summer was less intense than in spring, a total of 1298 eggs being recorded for the summer peak period (24 June-14 July) compared with 5796 eggs during a similar period (30 April-20 May) in spring.

Observations on the numbers of flies that emerged from alternate plants on the observation plot showed that the low rate of egg-laying in summer was not the result of a lack of adults. Consideration of biotic and climatic factors suggested that while high temperatures and long periods of sunlight stimulated the summer flies to great activity the environment provided little food to sustain them, and in consequence they did not survive to complete oviposition.

The puparia were collected from the undisturbed alternate plants in order to obtain information on the numbers of *E. brassicae* that reached maturity. It was estimated that 5-6 % of the eggs reached the pupal stage; approximately two-thirds of the puparia gave rise to flies and one-third were parasitized. About 9 % of the puparia remained viable in the soil to give rise to the following spring generation, about 90 % having given rise to flies in the year in which they were formed.

DURATION AND INTENSITY OF EGG-LAYING IN SPRING 1952

A small observation plot (10 × 10 ft.) was planted in March 1952 with twenty-four cabbages 2 ft. apart. The position of the 25th plant was occupied by a mercury-in-steel thermograph. The plot adjoined an allotment area where brassicas were cultivated all the year round, and a market crop of early cauliflowers grew approximately 100 yd. away. The soil from the bases of alternate plants was removed daily at about 9 a.m. (G.M.T.), and the eggs of *E. brassicae* were collected by flotation. The numbers of eggs were recorded as having been laid on the previous day. The duration of the spring egg-laying period and the numbers of eggs per day per plant are shown in Table 1.

The first eggs were found on 22 April, and in the week following only small numbers of eggs were laid. On 30 April the rate of egg-laying suddenly increased to twenty eggs per day per plant and except on one day (4 May) this rate was maintained for about a week. On 7 and 8 May the rate of egg-laying rose to more than

TABLE I. *Spring egg-laying by Erioischia brassicae*

Date examined	No. of eggs at plant nos.												Total no. eggs	Mean no. of eggs/plant/day (nearest integer)
	1	2	3	4	5	6	7	8	9	10	11	12		
22 Apr.	0	0	0	0	0	14	0	0	0	0	0	0	14	1
23 "	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 "	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 "	2	0	0	0	0	0	0	0	0	0	0	0	2	0
26 "	0	3	10	0	0	3	0	2	0	0	0	0	18	2
27 "	3	6	2	0	0	0	7	5	0	0	0	19	42	4
28 "	0	0	11	0	0	0	1	5	0	0	0	0	17	1
29 "	7	0	2	10	6	0	0	0	0	0	0	2	27	2
30 "	1	24	16	20	39	29	53	2	0	12	23	26	245	20
1 May	18	14	64	51	31	27	33	10	0	11	34	25	318	26
2 "	20	6	58	19	5	3	24	48	0	6	0	21	210	18
3 "	15	28	40	26	21	1	42	12	0	21	13	27	246	20
4 "	3	35	4	0	1	0	1	9	1	5	0	5	64	5
5 "	25	18	62	35	27	31	7	87	15	51	30	22	410	34
6 "	16	20	5	33	64	22	12	21	1	16	10	42	262	22
7 "	38	18	31	34	19	33	13	57	62	112	93	45	555	46
8 "	46	25	24	29	23	64	12	30	52	45	35	117	502	42
9 "	9	25	26	27	6	28	16	42	1	11	39	16	246	20
10 "	11	20	5	10	24	75	17	78	4	81	29	70	424	35
11 "	19	4	13	25	1	15	14	20	11	15	0	16	153	13
12 "	28	91	1	7	30	9	74	43	1	13	40	25	362	30
13 "	26	17	37	15	12	36	38	65	35	55	28	54	418	35
14 "	4	6	4	1	2	9	16	17	38	21	16	21	155	13
15 "	19	4	11	23	0	11	37	16	4	6	48	21	200	17
16 "	6	0	8	0	3	12	31	43	25	21	39	51	239	20
17 "	20	15	26	30	17	13	12	48	2	5	12	12	212	18
18 "	18	19	53	33	0	21	3	25	1	11	9	8	197	16
19 "	10	2	50	8	3	18	71	21	1	6	2	52	244	20
20 "	31	14	27	7	0	3	7	0	0	2	16	27	134	11
21 "	1	30	10	13	4	5	12	30	0	5	17	23	150	12
22 "	0	3	15	12	1	4	21	2	0	1	47	33	139	12
23 "	2	1	0	7	0	11	29	1	0	0	12	21	84	7
24 "	1	1	1	0	1	1	16	5	3	2	4	9	44	4
25 "	0	0	8	0	1	0	3	1	0	0	2	0	15	1
29 "	0	0	8	18	1	6	0	5	1	4	1	7	51	1
Total eggs/plant	395	449	632	493	342	504	622	750	258	538	599	817	6399	533 eggs/ plant

forty eggs per day per plant and then gradually declined. In the last week of May few eggs were laid, and it appeared that egg-laying by the spring generation had virtually ceased. By the end of May the plants under observation had received 258-817 eggs with a mean of 533 eggs per plant.

In 1952 the spring peak period of egg-laying lasted approximately 3 weeks (30 April–22 May), and during this time the plants under observation received eggs at the rate of 23 per day. The onset of infestation was sudden and occurred about a week after the first eggs were seen. It is not possible to say whether this is the normal pattern of infestation, because there appears to be no published records of the duration and intensity of egg-laying by *E. brassicae* in spring, but the infestation of the plants under observation appeared similar to that of the commercial and allotment crops growing nearby.

INFLUENCE OF WEATHER CONDITIONS ON THE RATE OF EGG-LAYING IN SPRING

Table 1 shows that during the spring egg-laying period the numbers of eggs per day per plant fluctuated. Fig. 1 shows the mean number of eggs per day per plant, the maximum day temperatures and the number of hours of sunshine per day. The

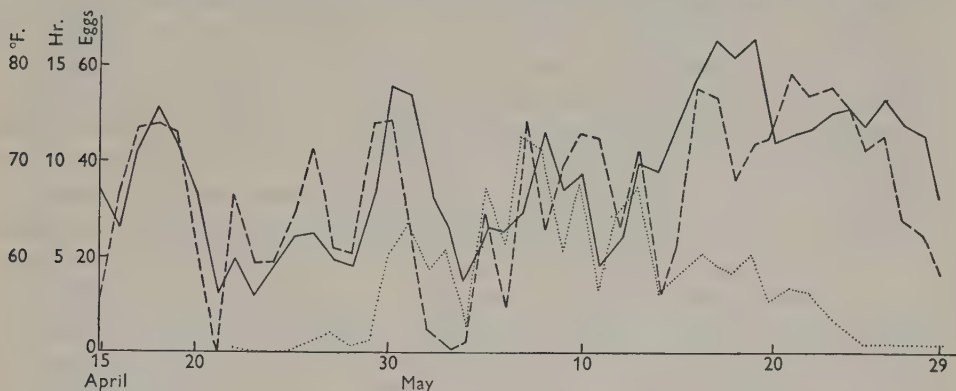


Fig. 1. Egg-laying by *E. brassicae*. Numbers of eggs per day per plant during spring egg-laying period., number of eggs. —, maximum temperature. ---, hours of sunshine.

temperatures given are those recorded on the mercury-in-steel thermograph which was placed 4 in. above the soil level. On cloudy days they differed little from the maximum screen temperatures recorded at the Wye meteorological station,* but on sunny days the temperature near soil level exceeded that recorded at the meteorological station by as much as 10° F.

Fig. 1 suggests that the daily rate of egg-laying by *E. brassicae* was affected by the temperature and sunshine. The peak period of egg-laying began on 30 April when there were 12 hr. of sunshine and the temperature rose to 78° F. On 4 May it rained almost all day, there was no sunshine, and the temperature fell to 57° F., and in these circumstances the rate of egg-laying fell to five eggs per plant. Better weather on the following day was associated with an immediate increase to thirty-four eggs per plant. The fall to twenty-two eggs per plant on 6 May was again associated with rain, but in the 2.3 hr. of sunshine the flies were sufficiently active to maintain the

* Less than 100 yards from the observation post.

rate of egg-laying at a high level. The fall in the rate of egg-laying on 11 May was associated with a fall in temperature, wind (Force 5, Beaufort scale) and some rain and cloud, and a similar fall in egg-laying occurred on 14 May which was cool and dull. During the third week of May the daily rate of egg-laying fluctuated with the temperature, but the decline in the numbers of eggs indicated that the spring peak was coming to an end. In the fourth week of May the rate of egg-laying was negligible, although the weather remained warm and sunny.

Life history of the first generation

The incubation period for the earliest eggs was 7 days, but in the high temperatures that prevailed in the latter half of May it was reduced to 2–3 days. Larval development was similarly affected, and at day temperatures of 70–85° F. larvae in captivity reached the pupal stage in 8–10 days and flies emerged 16–23 days later.

Data on the numbers of flies in the spring (April–May) and summer (June–July) generations were not collected, but it is general experience that mature larvae and puparia are more numerous and more easily found in May and early June than at other seasons, and when puparia of the spring generation are kept under observation flies emerge freely. The writer has recorded finding up to eighty-one larvae and puparia per plant at cauliflowers in June (Miles, 1950), but has rarely taken more than four to six viable puparia per plant at the roots of winter cabbage and savoy in February. This suggests that the number of flies emerging in June–July is greater than that emerging in April–May, and that summer infestations by *E. brassicae* should be quite as serious as those occurring in spring. Glasgow (1925) and Brittain (1927), in North America, have stated that only the first generation is of major economic importance, and Glasgow, who recognized that large numbers of *E. brassicae* emerged in early summer, concluded that the later generations were reduced by natural enemies or unfavourable environment. Observations (Miles, 1952) indicated that in Kent, also, the summer infestation by *E. brassicae* is less important than that occurring in spring.

EGG-LAYING BY *ERIOISCHIA BRASSICAE* IN SUMMER AND AUTUMN

The cabbages on the observation plot at Wye were allowed to grow undisturbed through the summer and autumn so that emerging flies could readily find host plants. The dry hot weather tended to check growth, and the plants remained rather small with leaves close to the ground, thus providing shelter similar to that afforded by field crops. There was no noticeable increase of brassica crops in the vicinity. Counts of eggs were made in the same manner as in spring and from the same plants, but they were done twice weekly in June, July and August and at weekly intervals in September and October. Data collected are given in Table 2.

Table 2 shows that the highest numbers of eggs were found in late June and early July during the expected emergence period of the summer generation. Comparison of Tables 1 and 2 shows that the daily rate of egg-laying in summer and autumn was

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much lower than it had been in spring. A total of 1298 eggs, or an average of five per day per plant, was recorded for the summer peak period (24 June–14 July), while for a similar period in spring (30 April–20 May) 5796 eggs, twenty-three per day per plant, were recorded. After mid-July the daily rate of egg-laying was so low as to be negligible.

TABLE 2. *Summer and autumn egg-laying by Erioischia brassicae*

Date	No. of eggs at plants nos.												Total no. eggs	Mean no. of eggs/day/plant
	1	2	3	4	5	6	7	8	9	10	11	12		
3 June	18	8	3	34	39	1	0	16	6	5	48	63	241	5
6 „	1	4	0	24	5	2	1	2	1	15	16	3	74	2
10 „	30	16	11	10	5	25	13	13	22	23	56	16	240	5
13 „	23	9	1	9	2	5	3	0	7	4	22	2	87	2
16 „	2	4	1	4	0	1	2	0	19	30	20	10	93	3
20 „	0	34	6	1	0	2	7	0	2	12	3	10	77	2
23 „	1	3	0	14	6	0	4	4	0	15	3	3	53	1
27 „	2	1	15	6	3	1	5	1	0	2	21	0	57	1
30 „	14	1	101	24	5	0	12	0	2	7	31	52	249	7
3 July	2	0	37	6	2	9	29	5	1	31	24	49	195	5
7 „	1	7	52	51	2	2	22	10	2	82	74	77	382	8
11 „	8	17	53	22	6	19	11	31	13	25	9	53	267	6
14 „	6	1	32	8	1	12	9	23	5	16	27	8	148	4
18 „	0	6	25	3	5	7	6	0	0	31	6	89	89	2
21 „	0	3	1	3	6	3	14	0	0	15	3	26	74	2
25 „	1	6	17	13	7	8	1	0	0	6	2	2	63	1
28 „	0	0	7	1	6	5	4	10	1	3	0	0	37	1
1 Aug.	0	1	10	2	7	10	0	44	2	0	24	20	120	2
8 „	0	2	3	4	3	0	7	5	5	30	13	2	74	1
11 „	0	2	5	1	1	0	0	4	0	3	1	0	17	0
15 „	5	0	5	12	0	1	1	2	0	3	5	0	34	1
18 „	3	1	1	5	1	1	0	8	1	3	0	2	26	1
22 „	11	0	4	1	0	1	4	9	0	0	0	1	31	1
29 „	8	0	0	11	0	1	5	7	0	17	13	14	76	1
6 Sept.	0	0	18	8	0	2	12	15	1	44	26	21	147	2
12 „	0	10	14	2	0	5	15	6	1	14	12	2	81	1
19 „	7	8	5	6	1	7	8	0	1	8	1	6	58	1
26 „	3	4	2	0	0	0	1	1	0	22	3	13	49	1
3 Oct.	0	2	0	1	0	2	1	1	1	14	1	1	24	0
10 „	1	7	2	1	0	0	8	0	0	27	4	2	52	1
17 „	6	0	1	2	1	1	1	2	8	12	0	1	35	0
24 „	0	0	1	0	0	0	1	0	0	8	0	0	10	0
31 „	0	1	0	2	0	0	0	0	0	0	0	0	3	0
Total	153	158	433	291	114	133	207	219	101	496	493	465	3263	

INFLUENCE OF WEATHER CONDITIONS ON THE SUMMER GENERATION

Fig. 2 shows the mean daily rates of egg-laying and the mean daily maximum temperatures for weekly periods from April to October. The maximum temperature is only one factor of the weather complex, but it has been used as an indicator because observations in spring showed its importance and also because several other factors are related to it. The curve for hours of sunshine tends to follow that for maximum temperature because the highest summer temperatures are usually

associated with bright sunshine. Temperature and air currents largely determine the relative humidity, and the curve of minimum relative humidity tends to follow inversely that of the maximum temperature. The effect of rain and the cumulative effect of drought on the habitat of *E. brassicae* are also mainly determined by temperature and sunshine.

Discussion of the rates of egg-laying is limited to the spring and summer egg-laying periods. In warm damp weather in late summer and early autumn *E. brassicae* often lay eggs among the foliage of the host plants. This habit may diminish the numbers of eggs laid in the soil in the latter part of the season, but it is unlikely to affect the relative importance of the several generations which form the annual life cycle.

Fig. 2 shows that most eggs were laid in spring while the average maximum temperatures were relatively low (i.e. below 70° F.) and that comparatively few eggs were found throughout the summer when the temperatures were persistently high.

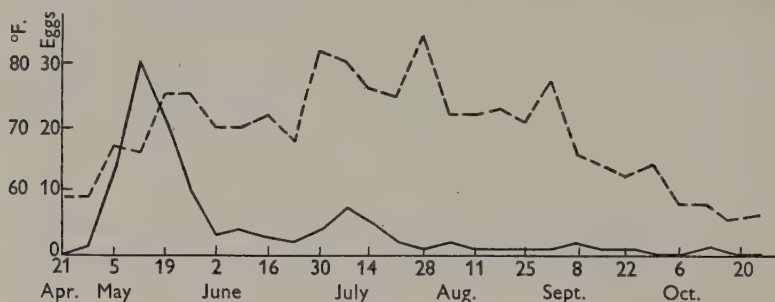


Fig. 2. Egg-laying by *E. brassicae*. Numbers of eggs per day per plant for weekly periods during April to October 1952. —, number of eggs. ---, average maximum temperature.

In the first 2 weeks of May when eggs were most numerous the weather was sunny (7 hr. sunshine a day) and showery (0.84 in. of rain). This suggests that mild sunny showery weather provides favourable conditions for *E. brassicae*. In the latter half of May the average maximum temperature was 75° F., there were 10 hr. of sunshine per day and no rain, and under these conditions the rate of egg-laying declined rapidly. Some of this decline was undoubtedly associated with the senescence and death of the flies, but it is possible that the decline might have been less rapid had conditions remained similar to those of the previous 2 weeks.

The small but distinct peak of egg-laying during June–July was associated with the emergence of the second generation of flies, but the numbers of eggs laid were much below those expected. The subsequent examination of the alternate plants on the observation plot showed that the low rate of egg-laying was not the result of a failure of adults to emerge. The cause was sought in the direct and indirect effects of weather conditions on the flies.

Females of *E. brassicae* emerge from the puparia with the oocytes in an early stage of development, and they do not mature unless the flies obtain suitable food. An

important source of food is nectar from flowers, and the writer has seen the flies sucking sap from broken stems of the host plants and, occasionally, moisture from soil. In 1952 when the summer generation was emerging, the mean maximum daily temperature was about 80° F. and the sun shone for approximately 11 hr. a day; there was rain on 3 days (total 0.54 in.), but droughty conditions prevailed. Nectar-producing flowers were scarce and short-lived; moisture about the plants evaporated quickly, and the rain made no lasting difference to the surface soil of the observation plot and the scorched herbage surrounding it. Under these conditions little food was available for the emerging flies.

Gibson & Treherne in Canada (1916) found that cabbage root flies emerging between 26 June and 1 July lived only 2–5 days, a duration of life much less than that of flies emerging at other times of the year. A consideration of climatic and biotic factors suggests that in south-east England the duration of life of the second generation which emerges during late June and early July, is similarly shortened. The long, hot days at this season stimulate the emerging flies to great activity and they are soon exhausted unless they have access to liquid food. It was shown (Miles, 1951) that flies emerging during 30 June–3 July and kept in captivity with plenty of food, lived up to 8 weeks. Under natural conditions liquid food is not easily accessible at this time and it seems probable that many flies die before the completion of egg-laying. This would account for the small numbers of eggs on the observation plot during the summer peak period of egg-laying.

NUMBERS OF *ERIOISCHIA BRASSICAE* REACHING MATURITY ON THE OBSERVATION PLOT

Infestation by *E. brassicae* was allowed to develop naturally on the remaining twelve plants on the observation plot. No observations were made on the numbers of eggs laid on these plants, but the respective numbers recorded from the adjacent plants nos. 1–12 during April–October (Tables 1 and 2) were: 548, 607, 1065, 784, 456, 637, 829, 969, 359, 1034, 1092 and 1282: a total of 9662 eggs. There was wide variation in the infestation of the several plants, but it was expected that the total number of eggs at one series of twelve plants would be approximately similar to that of a second series in alternate positions.

The undisturbed plants were lifted in late December, each with an 8 in. cube of soil containing practically all the root system. They were stored in open boxes in an insectary until late February and early March. The soil and plant roots were then washed over a 1.5 mm. sieve and puparia were separated from gravel and vegetable fragments by flotation. Viable and empty puparia floated readily, but it was thought that a small proportion of empty puparia may have become filled with silt during the washing process and were not recovered. Nearly all the puparia were found in close proximity to the plant roots:

The following numbers of viable and empty puparia of *E. brassicae* were recovered respectively from the twelve plants examined: 41, 27, 77, 37, 29, 26, 13, 138, 4,

33, 45 and 73; a total of 543 puparia. If it is accepted that the initial infestation of the two series of plants was similar then the 543 puparia represents 5-6% of the total number of eggs laid.

Examination showed that only thirty-three puparia contained viable pupae of *E. brassicae*, an average of less than three per plant. It is not known whether the overwintering generation was generally less numerous in 1952-3 than usual, but a factor that may have adversely affected the numerical strength of the autumn generation was the low temperature during September-October when the maximum temperature exceeded 60° F. on only 18 days as compared with an average of 38 days during the years 1948-51. The infestation potential of the overwintering puparia cannot be estimated because the ratio of sexes at this season is not known, but in no circumstances could the puparia collected on the observation plot give rise to an attack similar to that observed in spring 1952 (Table 1).

Of the 543 puparia recovered, 334 had developed normally and had given rise to flies in the course of the summer. These flies may be considered as having laid eggs found on the plot from the end of May onwards, i.e. those recorded in Table 2. Since each female is capable of laying about 100 eggs (Miles, 1951) it would appear that the small numbers of eggs in summer could not be ascribed to a paucity of flies.

A total of 150 puparia showed signs of parasitism. Fifteen of the parasitized puparia appeared to contain viable parasites. Since only thirty-three puparia contained viable pupae of *E. brassicae*, it appears that on the observation plot about one-third of the overwintering puparia were parasitized. The other 135 puparia showed the characteristic irregular holes, more or less complete, associated with the emergence of parasites and indicated that the rate of parasitism in spring and summer was also approximately 30%.

Eighteen puparia were intact but obviously empty. A further eight puparia were identified as those of *E. brassicae*, but they were too badly broken for any decision to be made regarding the insects that had emerged.

CONCLUSION

This study of egg-laying by *E. brassicae* throughout 1952 has shown that infestation was heaviest in spring, only slight at midsummer when the second generation was active, and negligible from mid-July onwards through the late summer and autumn. The onset of attack in spring, as shown by the daily rate of egg-laying, was encouraged by warm sunny weather; it developed rapidly and continued for about 3 weeks. This pattern of attack showed that control measures should be applied at the beginning of the egg-laying period if the greatest benefit was to be obtained from their use.

If, as suggested here, the intensity of attack by the summer generation is determined by weather conditions, it may vary from year to year. Hot, dry weather, which is unfavourable to the development of attack by *E. brassicae*, is also unfavourable to the establishment of newly set plants, and wilting and death of plants in

field crops in summer, usually ascribed to attack by *E. brassicae*, is accentuated by mechanical or other injury to the roots prior to setting out. Showery weather in summer favours attack by *E. brassicae*, but it also creates conditions which favour the early establishment of newly set plants. From observations at Wye it seems that in the south-east of England protective treatment against *E. brassicae* in summer is necessary only in exceptional circumstances.

This study of *E. brassicae* contributes to the better understanding of the incidence of attack by other injurious Anthomyiid flies, including the mangold fly (*Pegomyia betae* Curt.), the bean seed flies (*Delia cilicrura* Rond. and *D. trichodactyla* Rond.) and the spinach stem fly (*Hylemyia echinata* Ség.). These species are multivoltine with the spring and summer generations active simultaneously with those of *Erioischia brassicae*, and they also require food in liquid form to sustain them and to mature their eggs. In consequence, the intensity of their attacks is likely to be affected by weather conditions in the same way as that of *E. brassicae*. Observations at Wye have shown that the second and subsequent generations of mangold fly and spinach stem fly, like those of cabbage root fly, are generally of little economic importance. The erratic nature of spring attacks by bean seed flies, which are highly sensitive to weather conditions, can be explained by reference to the variation in the daily rate of egg-laying by *E. brassicae* during the spring peak of activity. It also seems probable that summer weather conditions determine the geographical distribution of the turnip fly, *E. floralis* Fall., which is mainly univoltine. It emerges from late June to early August and since it also requires liquid food it can persist only in areas where such food is readily accessible at that time.

The writer expresses thanks to the Principal and Governors of Wye College for the facilities afforded for the work and to the Royal Society for a grant which enabled her to obtain a mercury-in-steel thermograph and other apparatus. The help and criticism of Professor Herbert W. Miles is gratefully acknowledged, as also is the assistance of Miss D. Johns in the preparation of graphs for publication.

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BIOLOGICAL INVESTIGATIONS INTO THE VALIDITY OF *CONTARINIA* SPECIES LIVING ON THE CRUCIFERAE, WITH SPECIAL REFERENCE TO THE SWEDE MIDGE, *CONTARINIA NASTURTII* (KIEFFER)*

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(With Plate 6)

Biological investigations have proved that *Contarinia nasturtii* (Kieffer), *C. isatidis* Rübsaamen and *C. ruderalis* (Kieffer) are in reality one species. The names *C. isatidis* Rübsaamen and *C. ruderalis* (Kieffer) are therefore synonyms of *C. nasturtii* (Kieffer). Considerable doubt has been thrown on the authenticity of several other species.

The host plant range of *C. nasturtii* was already known to be very extensive. Fourteen additional host plants, including two tetraploid varieties and several weeds have been added during this investigation. Eight were established by experiments, and the remaining six have previously been recorded as host plants of *C. ruderalis*. In addition, *Brassica nigra*, *Lepidium sativum* and *Rapistrum rugosum* have since been established as host plants of *Contarinia nasturtii* in Holland. *Diplotaxis tenuifolia* and *Lunaria annua* are suspected as host plants, but the evidence is not yet conclusive.

INTRODUCTION AND METHODS

In the past gall midges of the same genus have often been described as distinct species if they occurred on different host plants. This was a useful precaution against confusing species in the absence of more detailed knowledge of the insects concerned. Certain gall-midge species are now known to have a range of host plants, and some show such a gradation in morphological characters when large numbers of specimens are examined that specific determination by the usual methods is difficult. This has led to the development of biological techniques in which studies of life histories, host-plant range and mating tests play a part.

A large number of midges must be available for such work. They can be bred from larvae in galls collected either in the field or from a plot where host plants are intensively grown. This may quickly become infested if the midges are in the vicinity and saves many hours of field searching. The plants can be examined frequently and the midges, bred out from collected galls in emergence cages, are available for biological tests.

Such a plot was established at Rothamsted Lodge, Harpenden, in an investigation of the genus *Contarinia* infesting the Cruciferae. Thirty-four different crucifers (marked with an asterisk in Table 1) were grown, of which ten were known host

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plants of *C. nasturtii* (Kieffer), three of *C. ruderalis* (Kieffer) and one each of *C. isatidis* Rübsaamen, *C. lepidii* Kieffer and *C. thlaspeos* Rübsaamen. The remaining eighteen plants were suspected or on trial as hosts. Similar plants were grown in pots under insect-proof cages so that experimental infestations could be made.

C. nasturtii, the swede midge, was known to have infested the plot previously, and soon became re-established. Material of this species was also collected from other localities, and some was kindly sent by D. C. Thomas, M. Cohen and W. C. Nijveldt from Devon, Cumberland and the Netherlands respectively, thus avoiding the possible disadvantages of using a purely local strain. *C. isatidis* and *C. ruderalis* also appeared on the plot. Biological experiments were made with these three midges, and with other undescribed *Contarinia* species, obtained from the plot and elsewhere in an attempt to establish their true specific identity.

The sources of the names of the crucifers mentioned are given in Table 1 and synonyms are given only where they are relevant to the literature.

CONTARINIA NASTURTII (KIEFFER)

Syn. *C. torquens* Meijere, *C. perniciosa* Rübsaamen, *C. geisenheyneri* Rübsaamen.

C. nasturtii, which was originally described from the swollen flower buds of a wild water-cress (Kieffer, 1888), was known at the beginning of this study to live in the flowers or on the leaves of sixteen other crucifer species (Table 2).

Cultivated varieties of these species included cabbage, cauliflower, savoy, rape, turnip, swede, cow-cabbage, edible water-cress and horse-radish (Pl. 6, fig. 2). Bovien & Knudsen (1950) and Barnes (1950) had proved biologically that the swede midge could damage both flowers and leaves.

Host-plant trials

Experimental infestations were made by placing midges on host plants grown in pots under muslin cages. Males and females from different sources were usually used, and where possible were placed to breed on plants in a different stage of growth from that on which they themselves were reared. Oviposition, the development of the gall and subsequent emergence of the new generation were observed.

Table 3 shows six new host plants, *Brassica alba*, *Brassicella wrightii*, *Sisymbrium officinale* (Pl. 6, fig. 4), *Thlaspi arvense* (Pl. 6, fig. 1), and the tetraploid varieties of *Brassica Napus* var. *oleifera* and *B. Rapa* var. *rapifera*. The first two experiments were of a confirmatory nature, using two varieties of *B. Rapa* in different stages of growth as host plants. The experiments (nos. 10 and 11) with the Colza midges showed that *C. nasturtii* from the Netherlands could also cause more than one type of damage. The existence of biological races restricted to one type of plant damage had long been in dispute. Further experiments on this point have since been conducted in Holland (Stokes, 1953). Another experiment, in which two males and one female of the new generation of midges bred in Exp. 10 were caged with *Sisymbrium officinale* showed they were also capable of breeding on this host plant.

TABLE I. *Table of plant names.*¹ Cruciferae

(An asterisk indicates plants grown during this investigation)

Name used in this paper	Source	Common name	Synonym
<i>Armoracia lapathifolia</i> Gilib.	B	Horse-radish	<i>Cochlearia armoracia</i> L.
* <i>Brassica alba</i> (L.) Boiss.	A	White mustard	—
<i>B. amplexicaulis</i> (Desf.) Pomel.	C	—	—
* <i>B. campestris</i> L. ²	A	Wild turnip	—
* <i>B. campestris</i> L. var. <i>chinensis</i>	A	Chinese cabbage, Pak choi	<i>B. chinensis</i> L.
(<i>B. cheiranthos</i> (Vill.) Pugsl.), see <i>Brassicella erucastrum</i>	—	—	—
(<i>Brassica chinensis</i> L.), see <i>B. campestris</i> var. <i>chinensis</i>	—	—	—
<i>B. fruticulosa</i> Cyrill	—	—	—
* <i>B. Napus</i> L.	A	Swede, swede-like rapes	—
* <i>B. Napus</i> L. var. <i>oleifera</i>	C	Colza	—
* <i>B. nigra</i> (L.) Koch	A	Black mustard	—
* <i>B. oleracea</i> L.	A	Cabbage, cauliflower, savoy, Shetland cabbage, marrow- stem kale	—
* <i>B. Rapa</i> L.	A	Turnip, turnip-like rapes	<i>B. rutabaga</i> L.
* <i>B. Rapa</i> L. var. <i>oleifera</i>	—	—	—
* <i>B. Rapa</i> L. var. <i>rapifera</i>	—	—	—
(<i>B. rutabaga</i> L.), see <i>B. Rapa</i>	—	—	—
(<i>B. sinapis</i> Vis.), see <i>B. sinapistrum</i>	—	—	—
* <i>B. sinapistrum</i> Boiss.	A	Charlock	<i>B. sinapis</i> Vis.
<i>Barbarea vulgaris</i> R. Br.	B	Yellow rocket, wintercress	—
<i>Brassicella erucastrum</i> (L.) O. E. Schulz., see Footnote on <i>Sinapis cheiranthoides</i> , p. 729	C	—	<i>B. cheiranthos</i> (Vill.) Pugsl. and <i>Sinapis Cheiranthus</i> (Vill.) Koch
* <i>Brassicella wrightii</i> O. E. Schulz	B	Lundy Island cabbage	—
* <i>Bunias orientalis</i> L.	B	—	—
<i>Camelina microcarpa</i> Andranz	—	—	—
(<i>Cochlearia armoracia</i> L.), see <i>Armoracia lapathifolia</i>	—	—	—
* <i>Descurainia sophia</i> (L.) Prantl	B	Flixweed	<i>Sisymbrium sophia</i> L.
* <i>Diplotaxis erucoides</i> (L.) DC.	C	—	—
* <i>D. tenuifolia</i> (L.) DC.	B	Narrow-leaved wall- mustard	—

¹ Names marked A are taken from the Imperial Bureau Joint Publication No. 5, 1943, *The Production of Seed of Root Crops and Vegetables*; those marked B from *Drawings of British Plants Part III, Cruciferae*, 1949, by Stella Ross-Craig; and those marked C from *Die Natürlichen Pflanzenfamilien*, Band 176, 1936, by Engler and Harms. The few exceptions not found in any of these classifications are given with the authorities in the form found in the midge literature. Relevant common names have been compiled from a variety of sources.

Authorities for plant names are not given in A. They have been ascertained from the original unpublished work which provided the basis of the *Brassica* classification in this publication. Permission for this was kindly given by the Director of the National Institute of Agricultural Botany.

² *B. campestris* is considered by some authorities to be the same species as *B. Rapa* but is kept separate here in order to distinguish it from cultivated forms of *B. Rapa*, e.g. turnip.

TABLE I (cont.)

Name used in this paper	Source	Common name	Synonym
* <i>Eruca sativa</i> Gars.	C	—	—
<i>Erucastrum incanum</i> Koch	—	—	—
<i>Erysimum cheiranthoides</i> L.	—	—	—
<i>Hirschfeldia incana</i> (L.) Lagreze-Fossat	C	—	<i>Sinapis incana</i> L.
* <i>Isatis tinctoria</i> L.	B	Dyer's woad	—
* <i>Lepidium draba</i> L.	B	Pepperwort, whitlow pepperwort, hoary cress	—
* <i>L. ruderale</i> L.	B	Apetalous pepperwort	—
* <i>L. sativum</i> L.	C	Common cress	—
* <i>L. virginicum</i> L.	C	—	—
* <i>Lunaria annua</i> L.	C	Honesty	—
(<i>Nasturtium amphibium</i> (L.) R. Br.), see <i>Rorippa amphibia</i>	—	—	—
<i>N. officinale</i> R.Br.	B	Common water-cress	—
(<i>N. palustre</i> (Leyss) DC.), see <i>Rorippa islandica</i>	—	—	—
<i>Nasturtium pyrenaicum</i> (L.) R.Br.	C	—	—
(<i>N. sylvestre</i> (L.) R.Br.), see <i>Rorippa sylvestris</i>	—	—	—
<i>Raphanus caudatus</i> L.	C	—	—
<i>R. raphanistrum</i> L.	B	Wild radish	<i>R. silvestris</i> Lam.
* <i>R. sativus</i> L.	C	Radish	—
(<i>R. silvestris</i> Lam.), see <i>R. raphanistrum</i>	—	—	—
* <i>Rapistrum rugosum</i> (L.) All.	C	—	—
* <i>Rorippa hybrid</i> ¹	—	—	—
<i>R. amphibia</i> (L.) Besser	B	Great yellow cress	<i>Nasturtium amphibium</i> (L.) R.Br.
<i>R. islandica</i> (Oeder) Borbas	B	Yellow cress	<i>Nasturtium palustre</i> (Leyss) DC.
<i>R. sylvestris</i> (L.) Besser	B	Creeping yellow cress	<i>Nasturtium sylvestre</i> (L.) R.Br.
<i>Sinapis cheiranthoides</i> ²	—	—	—
(<i>S. Cheiranthus</i> (Vill.) Koch), see <i>Brassicella erucastrum</i>	—	—	—
(<i>Sinapis incana</i> L.), see <i>Hirschfeldia incana</i>	—	—	—
<i>Sisymbrium altissimum</i> L.	B	Sisymbrium	<i>S. pannonicum</i> Jacq.
* <i>S. austriacum</i> Jacq.	C	—	—
(<i>S. columnae</i> Jacq.), see <i>S. orientale</i>	—	—	—
* <i>S. irio</i> L.	B	London rocket	—
<i>S. Loeselii</i> L.	C	—	—
* <i>S. officinale</i> (L.) Scop.	B	Hedge mustard	—
<i>S. orientale</i> L.	B	Sisymbrium	<i>S. columnae</i> Jacq.
(<i>S. pannonicum</i> Jacq.), see <i>S. altissimum</i>	—	—	—
(<i>S. sophia</i> L.), see <i>Descurainia sophia</i>	—	—	—
* <i>Thlaspi arvense</i> L.	B	Field penny-cress	—

¹ Identified at Kew as '?*Rorippa amphibia* (L.) Besser × *R. sylvestris* (L.) Besser'.

² Authorities at Kew were not able to trace the name *Sinapis cheiranthoides* and suggested it was used either for *Erysimum cheiranthoides* L. or *Brassicella erucastrum* (L.) O. E. Schulz (*Brassica cheiranthos* (Vill.) Pugsl.). Another synonym for *Brassicella erucastrum* is *Sinapis Cheiranthus* (Vill.) Koch.

TABLE 2. *The host plant range of Contarinia nasturtii*, 1888-1942

Date	Host plant name as recorded	Author	Present plant name
1888	<i>Nasturtium palustre</i> DC.	Kieffer	<i>Rorippa islandica</i> (Oeder) Borbas
1890	<i>Raphanus caudatus</i> L.	Kieffer	<i>Raphanus caudatus</i> L.
1893	<i>Brassica</i> spp.	—	<i>Brassica</i> spp.
	<i>Raphanus raphanistrum</i> L.	Kieffer	<i>Raphanus raphanistrum</i> L.
1898	<i>Nasturtium silvestre</i> (L.) R.Br.	Kieffer	<i>Rorippa sylvestris</i> (L.) Besser
	<i>Raphanus sativus</i> L.	Kieffer	<i>Raphanus sativus</i> L.
	<i>Brassica Napus</i> L.	Kieffer	<i>Brassica Napus</i> L.
	<i>B. oleracea</i> L.	Kieffer	<i>B. oleracea</i> L.
	<i>Sinapis cheiranthoides</i>	Kieffer	—
1903	<i>S. Cheiranthus</i> Mert & Koch	Kertesz	<i>Brassicella erucastrum</i> (L.) O. E. Schulz
1905	<i>Nasturtium pyrenaicum</i> L.	Pierre	<i>Nasturtium pyrenaicum</i> (L.) R.Br.
1906	<i>Brassica amplexicaulis</i> Janka	Stefani-Perez	<i>B. amplexicaulis</i> (Desf.) Pomel
1912	<i>B. rutabaga</i> L.	Taylor	<i>B. Napus</i> L.
1915	<i>B. Napus</i> (turnip)	Dry	<i>B. Rapa</i> L.
	<i>B. Rapa</i> (rape)	Dry	<i>B. Rapa</i> L.
	<i>B. sinapis</i>	Dry	<i>B. sinapistrum</i> Boiss.
1925	<i>Nasturtium officinale</i> R.Br.	Rübsaamen & Heddicke	<i>N. officinale</i> R.Br.
1939	<i>N. amphibium</i> (L.)	Rübsaamen & Heddicke	<i>Rorippa amphibia</i> (L.) Besser
1942	<i>Cochlearia armoracia</i> L.	Noll, Roesler & Benner	<i>Armoracia lapathifolia</i> Gilib.

TABLE 3. *Contarinia nasturtii* bred experimentally on different host plants

Exp. no.	Host plant and gall of parent midges		Experimental host plant	Part of plant galled
1	1-0* Radish flowers	× 0-1 Radish flowers and 0-1 Swede leaves	<i>Brassica Rapa</i> var. <i>oleifera</i>	Flowers
2	1-0 <i>Rorippa</i> hybrid flowers	× 0-1 <i>B. Napus</i> var. <i>oleifera</i> inflorescence	<i>B. Rapa</i>	Leaves
3	1-0 Rape shoots and 1-0 Swede shoots	× 0-3 Radish flowers 0-1 Rape shoots 0-1 Swede shoots	<i>B. alba</i>	Flowers
4	1-0 Swede leaves	× 0-2 Swede leaves	<i>Sisymbrium officinale</i>	Flower buds
5	2-0 <i>Contarinia nasturtii</i> Source unspecified	× 0-4 <i>C. nasturtii</i> Source unspecified	Tetraploid <i>Brassica Napus</i> var. <i>oleifera</i>	Flowers
6	2-0 Turnip inflorescence axis	× 0-2 Swede leaves	Tetraploid <i>B. Rapa</i> var. <i>rapifera</i>	Leaves
7	1-0 <i>Rorippa</i> hybrid flowers	× 0-2 Radish flowers	<i>B. alba</i>	Flowers
8	2-0 Radish flowers	× 0-4 Radish flowers	<i>Thlaspi arvense</i>	Axillary buds and flower buds
9	1-0 Swede shoots	× 0-1 Rape shoots	<i>Brassicella wrightii</i>	Leaves and shoots
10	3-0 Colza shoots (Netherlands)	× 0-6 Colza shoots (Netherlands)	<i>Raphanus sativus</i>	Flowers
11	3-0 Colza shoots (Netherlands)	× 0-8 Colza shoots (Netherlands)	<i>Brassica alba</i>	Flowers

* Figures written thus 1-2 indicate one male and two female midges. This method has been adopted throughout the tables.

Further host plant trials were set up using *Contarinia nasturtii* bred on *Brassica alba* in Exp. 3 mated to new wild stock. *Eruca sativa* was shown to be another new host plant (Pl. 6, fig. 3), while *Raphanus sativus*, *Brassica Napus*, *B. sinapistrum* (charlock) and *Thlaspi arvense* were confirmed as host plants (Table 4). A description of the type of gall found on *Brassica sinapistrum* is not given in the literature. In Exp. 19 flower galls were found, but later inflorescence and leaf axil galls were also discovered (Pl. 6, fig. 5).

Contarinia nasturtii failed to establish families in experimental pots of *Sisymbrium irio*, *Isatis tinctoria* and *Lepidium sativum*, but these plants were found galled by

TABLE 4. *Contarinia nasturtii* bred experimentally on *Brassica alba* crossed with wild *Contarinia nasturtii*

Exp. no.	Host plant and gall of parent midges		Experimental host plant	Part of plant galled
16	1-0 <i>Brassica alba</i> flowers	× 0-1 <i>Raphanus sativus</i> flowers	<i>Eruca sativa</i>	Flowers
17	1-0 <i>B. alba</i> flowers	× 0-1 <i>Rorippa</i> hybrid flowers	<i>Thlaspi arvense</i>	Flowers
18	1-0 <i>B. alba</i> flowers	× 0-1 <i>Brassica</i> sp. (rape) shoots	<i>Raphanus sativus</i>	Flowers
19	1-0 <i>B. alba</i> flowers	× 0-1 <i>Brassica</i> sp.	<i>Brassica sinapistrum</i>	Flowers
20	1-0 <i>B. alba</i> flowers	× 0-1 <i>B. alba</i> flowers	<i>B. Napus</i> (swede)	Leaves

TABLE 5. *English Contarinia nasturtii* cross-mated with Netherlands *C. nasturtii*

Exp. no.	Host plant and gall of parent midges		Experimental host plant	Observations
12	1-0 Netherlands Colza shoot	× 0-1 <i>Rorippa</i> hybrid flowers	Swede leaves	Mating seen, no family
13	1-0 Netherlands Colza shoot	× 0-1 Mustard flowers	Radish shoots	Mating seen, no family
14	1-0 Netherlands Colza shoot	× 0-1 <i>Thlaspi arvense</i> flowers	Chinese cabbage	Mating seen, no family
15	1-0 Netherlands Colza shoot	× 0-1 Horse-radish flowers	—	Both midges escaped after mating

Contarinia larvae when grown outside. The species of midge responsible is considered later.

Mating experiments

It had been shown previously (Barnes, 1950) that *C. nasturtii* from *Rorippa* flowers mated with Netherlands *Contarinia nasturtii* also from *Rorippa*. During the present investigation mating between English *Contarinia nasturtii* from various plants and Netherlands *C. nasturtii* from Colza shoots occurred quite readily (Table 5).

Experiments were made to see if *C. nasturtii* would mate with other midges of the genus *Contarinia* described from other crucifers.

C. isatidis Rübsaamen, the woad midge, was found to be morphologically similar to *C. nasturtii*, and attempts were made to cross it with *C. nasturtii* and raise the resulting families on different host plants. Mating occurred readily and the offspring were reared on *Brassica Napus* and *Sisymbrium officinale* (Table 6).

There are several points of interest in Table 6. In Exps. 21 and 22 the female midges were *Contarinia nasturtii*, one from Devon, the other from Cumberland, and both laid eggs on a known host plant of *C. nasturtii*, *Brassica Napus* (swede), but the one was fertilized by *Contarinia isatidis* bred from a leaf gall, while the other by *C. isatidis* bred from a flower gall.

In contrast, the female midge used in Exp. 23 was *C. isatidis* from a leaf. The male was *C. nasturtii* from an inflorescence, and the eggs were laid on *Sisymbrium officinale* which is the normal host of *Contarinia ruderalis* and which, in Exp. 4, was found to support *C. nasturtii*.

TABLE 6. *Contarinia nasturtii* crossed with *C. isatidis*

Exp. no.	Host plant and gall of parent midges	Experimental host plant	Part of plant galled
21	1-0 <i>Contarinia isatidis</i> × 0-1 <i>C. nasturtii</i> (Chinese cabbage leaf shoot) Devon	<i>Brassica Napus</i>	Leaf
22	2-0 <i>C. isatidis</i> (flower) × 0-1 <i>C. nasturtii</i> (rape shoot) Cumberland	<i>B. Napus</i>	Leaf
23	2-0 <i>C. nasturtii</i> (Chinese cabbage inflorescence) × 0-4 <i>C. isatidis</i> (leaf)	<i>Sisymbrium officinale</i>	Inflorescence

Experimental evidence was also obtained that mating takes place between *C. nasturtii* and *C. ruderalis*, another very similar midge with a host plant range among species of *Sisymbrium*. In one experiment, in which a male of *C. ruderalis* bred from *Sisymbrium officinale* mated with a female *Contarinia nasturtii*, a new generation was raised from the leaves of *Isatis tinctoria*, the host plant of *Contarinia isatidis*.

Lastly, some unnamed midges belonging to the genus *Contarinia* were bred from galls of other cruciferous plants (Table 7). They appeared very similar to *C. nasturtii* and mated very readily with this midge. The cross between *C. nasturtii* and the midge from *Brassicella wrightii* resulted in a first generation being bred in radish flower buds. After a cross between *Contarinia nasturtii* and a midge from *Lepidium sativum*, galls were formed on *Brassica campestris* flower buds.

CONTARINIA ISATIDIS RÜBSAAMEN

This is the only gall midge that has been recorded from *Isatis tinctoria* L. or dyer's woad. The female was described by Rübsaamen (1910) who found galls in Germany. Apparently it has not been found elsewhere. A comparison with the description of the female of *Contarinia nasturtii* does not show any considerable morphological

difference. The male remains undescribed, but specimens reared during this investigation are also very similar to the male of *C. nasturtii*.

Records of the type of damage vary. In the original description the larvae are said to live on the undersides of the leaves and cause irregular protrusions, more or less discoloured, crumpled leaves and thickened veins which may split at the swollen end. Houard (1908-13) states that the stem is shortened and crowned with a little bunch of leaves, the petioles are enlarged at the base and more hairy than usual.

Isatis tinctoria was planted in the crucifer plot at Rothamsted Lodge and soon became infested with *Contarinia isatidis*. Larvae were found among crinkled veins and crumpled leaf tissue of a crisp texture (Pl. 6, fig. 6). This type of damage has also been found on woad in the Botanic Gardens of Cambridge University.

TABLE 7. *Contarinia nasturtii* crossed with *Contarinia* species from other cruciferous plants

Exp. no.	Host plant of parent midges		Experimental host plant	Observation
24	2-0 <i>Contarinia</i> sp. × 0-2 <i>C. nasturtii</i>	Exp. 9	Radish with flower buds	New generation bred
	<i>Brassicella wrightii</i>			
25	1-0 <i>Contarinia</i> sp. × 0-1 <i>C. nasturtii</i>	Chinese cabbage	<i>Brassica campestris</i> with flower buds	Flower galls seen
	<i>Lepidium sativum</i>			
26	1-0 <i>C. nasturtii</i> × 0-1 <i>Contarinia</i> sp.	<i>Eruca sativa</i>	Swede leaves	Mating seen, but no further developments
	<i>Rorippa</i> sp.			
27	1-0 <i>Contarinia</i> sp. × 0-1 <i>C. nasturtii</i>	Rape	Swede leaves	Mating seen, but spider killed in cage later. Another ♂ from <i>Rapistrum rugosum</i> and another ♀ <i>C. nasturtii</i> added. No developments
	<i>Rapistrum rugosum</i>			
28	1-0 <i>C. nasturtii</i> × 0-1 <i>Contarinia</i> sp.	<i>Eruca sativa</i>	Swede leaves	Mating seen, but no further developments
	Netherlands Colza			

Side shoots of the woad plants on the experimental plot were also galled. In this case the small leaves had swollen bases, some of which were tinged with purple and slightly crinkled. The small greenish purple flower buds developing in the centre were clustered together on an extremely short inflorescence axis. Galls of this type have also been found in a private garden at Bedford.

A third type of gall appeared on the plants in the plot. Closed and swollen individual woad flowers containing larvae appeared which were very similar to the galls of *C. nasturtii* on *Rorippa amphibia*. No previous record of *Contarinia* larvae living in woad flowers had been found. The larvae in each type of gall varied from greenish white to a yellowish colour.

Experiments

Midges reared from woad leaf galls bred successfully on *Brassica Napus* (swede), where the larvae caused crinkled leaves. Midges from woad flower galls established a new generation on *Eruca sativa*, causing a closed, green, swollen flower-bud gall. Neither of these plants has been previously recorded as supporting *Contarinia isatidis*, although both were known to be host plants of *C. nasturtii*.

Mating took place between *C. isatidis* and *C. nasturtii* (see Table 6) and the resultant generations were reared on *Brassica Napus* and *Sisymbrium officinale*, a host plant of another midge, *Contarinia ruderalis*. *C. isatidis* females were also mated with *C. ruderalis* and the succeeding generation developed successfully on the inflorescence of *Sisymbrium officinale*.

CONTARINIA RUDERALIS (KIEFFER)

This gall midge was first bred by Kieffer (1890) from the galled inflorescences of *Sisymbrium officinale* (L.) Scop. (hedge mustard). Similar galls had previously been reported from *Descurainia sophia* (syn. *Sisymbrium sophia*). Rübsaamen (1895) attributed to *Contarinia ruderalis* galls on *Sisymbrium orientale* (syn. *S. columnae*), *S. Loeselii* and *S. altissimum* (syn. *S. pannonicum*); and Molliard (1895) added *S. irio* to the list. Kieffer (1901) reviewed the position, stating that *S. officinale*, *S. orientale* and *Descurainia sophia* were galled by *Contarinia ruderalis* while *Sisymbrium Loeselii* was galled by both a *Contarinia* and a *Dasyneura* species. Hellwig (1901) added *Camelina microcarpa* as a host plant. Houard's list (1908-13) differed in giving *Sisymbrium officinale*, *S. irio* and *Descurainia sophia* definitely as host plants, and *Sisymbrium Loeselii*, *S. orientale*, *S. altissimum* and *Camelina microcarpa* as probable hosts of the midge.

In each case the gall was reported as affecting the inflorescence. The flowering head became a spongy mass with thickened peduncles, and individual flowers were shortened and crowded together. In *Sisymbrium irio* the gall was described as pineapple shaped, with conical flower buds, and in *S. orientale* spherical, with thickened petals and stamens, enlarged sepals and a hairy calyx. In addition, malformed stems were found in *Descurainia sophia*. The lateral shoots, being stopped in their development, became rounded, green or red, and thickened, with enlarged and hypertrophied axillary leaves at their base. Damage to *Sisymbrium orientale* was similar. The midge has been reported from England, France, Germany, Italy, Russia and Transcaucasus.

Experiments

The midge established itself quickly on *Sisymbrium officinale* grown on the crucifer plot, causing clustered inflorescences, shortened peduncles, thickened stems, swollen, crinkled leaf axils sometimes tinged with purple, and crinkled leaves and veins. *S. irio* similarly showed crinkled leaves and swollen individual flowers. The midge also infested *S. altissimum* found on a factory dump in Luton,

Bedfordshire, causing thickened and clustered flowering shoots. The larvae varied in colour from white to yellowish.

In experiments *Contarinia ruderalis* was found to breed on *Brassica oleracea* (marrow-stem kale), one of the host plants of *Contarinia nasturtii*. As mentioned previously, specimens of *C. nasturtii* and *C. ruderalis* were seen to mate and rear offspring on *Isatis tinctoria*, a host plant of *Contarinia isatidis*. *C. ruderalis* also mated with *C. isatidis* and produced a generation on *Sisymbrium officinale*, now found to be a host plant of all three midges.

THE OCCURRENCE OF UNISEXUAL FAMILIES

It will be seen from the foregoing experiments that *Contarinia nasturtii*, *C. isatidis* and *C. ruderalis* are very closely related. Observations on their individual biologies bear out their similarity. A special point of interest is the occurrence of unisexual families. This phenomenon was first noticed in *C. nasturtii* by Barnes (1950). It was confirmed in the present investigation. In fifteen experiments in which midges of only one sex were bred, eight arose from the use of a solitary pair of midges in each experiment (Table 8). In no experiment in which a single pair of midges was used did both sexes arise.

TABLE 8. *The occurrence of unisexual families*

Parent midges used	♂ ♀	Sex of offspring	
		♂	♀
<i>C. nasturtii</i>	1-1	0-	76
	1-1	90-	0
	1-1	0-	23
	1-1	54-	0
	1-1	0-	35
	1-1	53-	0
<i>C. isatidis</i>	1-1	0-	25
<i>C. isatidis</i> × <i>C. nasturtii</i>	2-1	0-	67
<i>C. nasturtii</i>	1-2	0-	20
	1-2	31-	0
	2-2	0-	49
	1-2	0-	65
	2-4	0-	102
<i>C. ruderalis</i> × <i>C. nasturtii</i>	2-2	0-	66
<i>C. ruderalis</i>	1-2	0-	45

DISCUSSION

There is no reason to doubt that the *Sisymbrium* and woad midges found at Rothamsted are *Contarinia ruderalis* and *C. isatidis*, as described by Kieffer and Rübsaamen respectively. *C. ruderalis* is listed as a British insect and one of its host plants is a common weed. *C. isatidis* was not previously known as a British insect, but neither of the midges occurring at Rothamsted differs from the written descriptions either morphologically or biologically as far as can be ascertained. Should

a midge appear in some isolated place, host specific to *Isatis tinctoria*, and showing morphological differences, the whole problem would have to be reconsidered, but as yet there is no evidence that such populations exist.

There was no reason to connect *Contarinia nasturtii*, *C. isatidis* and *C. ruderalis* with each other at the time of their original descriptions, especially as their host plants and types of gall were different. Even when Rübsaamen & Hedicke (1925-39) later described the midges on a more comparable basis, the known galls and host plants were distinct. The extensive host plant range of *C. nasturtii* and the capacity to make more than one type of gall were in the early stages of discovery. The complexities surrounding the identity of *C. nasturtii* are well illustrated by the fact that three species, viz. *C. torquens*, *C. geisenheyneri* and *C. perniciosa* had already been considered to be synonymous.

The delimiting of species and genera in the Cruciferae is notoriously difficult, and it seemed probable that a midge already infesting a number of them would also be found on others within so closely related a group. Thus the possibility of an even wider host-plant range and further synonyms is strengthened.

C. nasturtii, *C. isatidis* and *C. ruderalis* show the ability to live on more than one host plant and share some in common. The full host plant range would probably take several more years to discover, but already it is seen to be wide. Each midge can cause more than one type of gall. Fertile cross-matings occur readily under experimental conditions. Their general biologies, season of activity and rate of development are similar.

Negative evidence must also be considered. Some experiments were set up which gave no results. The causes of failure are not easy to determine and may range from mistakes in technique to fundamental facts of midge biology. Those giving negative results should be repeated numerous times before they are accepted as true failures. A positive result, on the other hand, is good evidence towards a conclusion, and repetition ensures it is not an exceptional case.

The results of this investigation indicate that *C. isatidis* and *C. ruderalis* are not valid species; they cannot be distinguished on morphological grounds and are biologically inseparable from *C. nasturtii*. As *C. nasturtii* (Kieffer) was described first this name must be maintained, and *C. isatidis* Rübsaamen and *C. ruderalis* (Kieffer) must be relegated to synonymy.

The newly discovered host plants of *C. nasturtii* therefore now include those of *C. isatidis* and *C. ruderalis* and, in addition, the plants on which the midge was found to breed in the course of this investigation. The full additional list is *Brassica alba* (L.) Boiss., tetraploid *B. Napus* L. var. *oleifera*, tetraploid *B. Rapa* L. var. *rapifera*, *Brassicella wrightii* O. E. Schulz, *Thlaspi arvense* L., *Eruca sativa* Gars., *Isatis tinctoria* L., *Sisymbrium officinale* (L.) Scop., *S. orientale* L., *S. Loeselii* L., *S. altissimum* L., *S. irio* L., *Descurainia sophia* (L.) Prantl, and *Camelina microcarpa* Andranz. Nothing is yet known of the relative intensity of the attack on different host plants. For practical purposes, it is seen that several common weeds may act

as reservoirs for *Contarinia nasturtii* and must be considered in attempts to control the midge.

CONTARINIA LEPIDII KIEFFER

Rübsaamen (1895) wrote an account of the deformation of the growing point of *Lepidium draba* L., in which he found larvae, later named *Contarinia lepidii* by Kieffer (1909).

The same midge was stated to have been found in England on *Lepidium sativum* (Barnes, 1926). But this identification must be held in doubt as subsequent examination of the preserved gall showed it to be an inflorescence gall and the host plant differs from the original one.

The midge itself remains unknown and undescribed, being named only from the gall and larvae.

In this investigation attempts were made to find the original type of damage on *L. draba*, but without success. A male midge was bred from the inflorescence of *L. sativum* found on the Rothamsted Lodge crucifer plot and mated with *Contarinia nasturtii* before being preserved in alcohol. The female midge was then caged with *Brassica campestris* and flower galls developed. No midges emerged, possibly because the experiment was set up late in the season. *Lepidium sativum* was subsequently shown in Holland to be a host plant of *Contarinia nasturtii* (Stokes, 1953) (Pl. 6, fig. 8).

CONTARINIA THLASPEOS RÜBSAAMEN

This midge emerged from the slightly swollen silicles of *Thlaspi arvense* L. (Rübsaamen, 1910). It is exceptional for a *Contarinia* species to be reported from cruciferous seed-pods, although it is a typical habitat for *Dasyneura* larvae, e.g. *D. brassicae* Winnertz. Rübsaamen also bred a gall midge of unusually large size and with only three-segmented palps instead of four as in *Contarinia*, but as it conformed to *Contarinia* in other characteristics he named it *C. thlaspeos* var. *major*.

Thlaspi arvense was grown on the crucifer plot at Rothamsted Lodge, but was not seen to be infested by any midges, although *Contarinia nasturtii* was found to breed successfully on its flower buds in experiments. Until specimens of the *Contarinia* from *Thlaspi arvense* are bred from galls collected in the field, no further progress can be made in the investigation of *Contarinia thlaspeos*.

CONTARINIA GALLAICA TAVARES, *C. PONTEVEDRENSIS* TAVARES AND
C. TUDENSIS TAVARES

The descriptions of these three gall midges (Tavares, 1916*a, b*) are the most detailed of any relating to *Contarinia* species on crucifers, nevertheless their use is limited in many respects. Tavares attempted to distinguish his new species on morphological grounds alone, without considering biological data. *C. tudensis* was found on *Erucastrum incanum*, *Contarinia pontevedrensis* bred on both *Raphanus raphanistrum*

(syn. *R. silvestris*) and *Brassica Napus*, and *Contarinia gallaica* on all three plants. Both *Raphanus raphanistrum* and *Brassica Napus* are host plants of *Contarinia nasturtii*. Tavares considered his three species were closely related to this midge, to *C. ruderalis* and to each other on morphological grounds. He attempted comparisons, but found that the characters he considered important, for example the measurement of palp segments, were not given in the original descriptions. He apparently had no specimens of *C. nasturtii* or *C. ruderalis* to examine for himself.

His own specific descriptions were first of size, colour and shape, followed by numerical or proportional details of different characters. But no indication of the number of specimens examined was given, therefore it is not possible to assess the true value of his method. He was well aware of his difficulties, commenting on them several times. He described *C. tudensis* from the female only, saying he could distinguish it better from *C. nasturtii* and *C. ruderalis* when the male, pupa and larva were known.

Biological tests for the validity of Tavares's species would be extremely difficult, because midges bred from the galls (which he said were indistinguishable) could not be identified without microscopic examination. A study of morphological variation among the *Contarinia* species on crucifers would be the best method of clarifying the situation.

CONTARINIA KIEFFERI (SCHLECHTENDAL)

This midge has never been described. The name was given to the larvae in a gall on *Descurainia sophia* (syn. *Sisymbrium sophia*) recorded by Schlechtendal (1891) in Germany. The typical *Contarinia* larvae lived among swollen bracts on the main or lateral shoots, making with the stunted laterals a roundish, fleshy, green or reddish gall.

C. ruderalis has been recorded from the same host plant. It is possible, though not determinable, that this gall is also caused by *C. ruderalis*. This case illustrated the deplorable practice of giving specific names to larvae in galls.

UNDESCRIBED *CONTARINIA* SPECIES

Galls on *Diplotaxis tenuifolia* were reported by Hieronymus (1890). Swollen flower buds which remained closed, with the corolla and stamens shortened and thickened at the base, contained several whitish yellow Cecidomyid larvae. They were found in the Colosseum in Rome by Ascherson. Cecconi (1904) identified the larvae as *Contarinia* on finding further specimens. Similar galls, but with yellow *Contarinia* larvae, were found in Luton on *Diplotaxis tenuifolia*. Three female midges were bred from these. With so little information it is not possible to say whether the midges in the Colosseum, where wild plants were growing in 1890, were the same species as those living among the weeds of a Luton factory dump in 1951.

Kieffer (1895) found swollen flower galls on *Barbarea vulgaris* which suggested those of *Contarinia nasturtii* on *Rorippa* spp., rather than the oval galls of *Dasyneura*

sisymbrii commonly found on this plant. Midges of the genus *Contarinia* were bred from them (Kieffer, 1901), but no further information was given.

A *Contarinia* species has been reported from swollen flower galls of *Hirschfeldia incana* (syn. *Sinapis incana*) by Houard (1908-13) who quoted Cotte (1912) as the source of the information. The larvae were yellow. There is no record that midges were bred and nothing further is known.

Midges in the genus have also been reared from galled flowers of *Brassica fruticulosa* Cyrill subsp. *radicata* Dsf. in Algeria, and from a *Sinapis* sp. in Russia. Specimens of both these rearings are in the Barnes collection.

Contarinia females were observed egg-laying on the flowers of *Brassica alba* growing on the crucifer plot. This plant was not then known to support any midges. Three were caught alive and each was caged with a different plant. One was placed on *B. Napus* (swede), one on *B. Rapa* (turnip) (both hosts of *Contarinia nasturtii*) and the third on *Isatis tinctoria*. The plants were flowering. Each of these midges oviposited in the flower buds of their new host plants, and the new generations developed successfully. Continuing the experiment with the offspring, a male was taken from the *Brassica Napus* experimental pot and a female from the *B. Rapa* pot. These mated readily and were caged with *Sisymbrium officinale*, a host plant of *Contarinia ruderalis*. The development of leaf and galls was seen, and a further generation of midges emerged. As stated previously, *Brassica alba* itself was found by experiment to be a host plant of *Contarinia nasturtii* though this was not known when the wild *Contarinia* species was caught on the flowers. There can be little doubt now that this midge on *Brassica alba* was *Contarinia nasturtii*. Similar specimens were also bred from flower galls of *Brassica alba* collected at Seale Hayne, Devon. Three female midges emerged but were not used in biological work.

Inflorescence galls from the tetraploid *B. Napus* var. *oleifera* containing *Contarinia* larvae were collected from the crucifer plot. Two male and two female midges emerged and were made into permanent slides. They appear to be *Contarinia nasturtii*. No records of midges on this host plant have been found, but it has been proved experimentally in the course of this investigation to be a host plant of *C. nasturtii* (Exp. 5). The diploid is also a host plant.

A yellow, closed flower gall on *Brassica nigra* was also found. No midges were bred and this plant was not used experimentally in host-plant trials. Subsequent experiments in Holland have shown it to be a host plant of *Contarinia nasturtii* (Stokes, 1953).

Brassicella wrightii (Lundy Island Cabbage) was also grown on the crucifer plot. Although no record of infestation by midges was found in the literature, H. G. Morgan has reported that he found leaf damage which he attributed to *Contarinia nasturtii* on this plant on Lundy Island in 1947 or 1948. E. D. Wiggins, who was on Lundy Island with him, noticed attacked plants in a garden in Sussex in 1949. H. F. Barnes also saw similar damage to the plants in Cambridge University Botanic Gardens in 1950. Crinkled leaves and *Contarinia* larvae on *Brassicella wrightii* were

found on the Rothamsted Lodge crucifer plot in 1951. Midges were bred out and mated with *Contarinia nasturtii*. A new generation was reared on radish. There can be no doubt that this wild midge from *Brassicella wrightii* was *Contarinia nasturtii*. *Brassicella wrightii* was found by experiment to be a host plant of *Contarinia nasturtii*.

Eruca sativa was also found galled by *Contarinia* larvae on the crucifer plot. The midges bred out mated with *C. nasturtii*, though no offspring were raised. The wild midge was very probably *C. nasturtii* since *Eruca sativa* was used successfully as a host plant for *Contarinia nasturtii*.

Crinkled leaves and pale yellow *Contarinia* larvae were found on *Lunaria annua* (Honesty) on the crucifer plot. Midges were reared successfully in 1952 but no biological tests were made. This is the first ornamental crucifer recorded as attacked by a species of *Contarinia*.

Rapistrum rugosum, an alien weed, was grown on the crucifer plot. Closed flower galls with *Contarinia* larvae were found and midges were reared. One mated with *C. nasturtii* in an experiment, but a spider probably accounted for the subsequent failure to rear offspring. Further tests are needed to establish its identity for certain, but it is highly probable that it is *C. nasturtii* since *Rapistrum rugosum* has been found to be a host plant of *Contarinia nasturtii* in Holland during 1952.

The author acknowledges with gratitude the encouragement and valuable criticism of Dr H. F. Barnes during the course of this investigation; and the many helpful suggestions of Prof. C. H. O'Donoghue of Reading University. The author is also indebted to Dr C. B. Williams for permission to submit the work as a thesis while working at Rothamsted Experimental Station. Grateful thanks are also due to Mr V. Stansfield for the photographs.

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EXPLANATION OF PLATE 6

- Fig. 1. Inflorescence and leaf axil gall of *Contarinia nasturtii* on *Thlaspi arvense* (penny-cress).
- Fig. 2. Flower gall of *Contarinia nasturtii* on *Armoracia lapathifolia* (horse-radish).
- Fig. 3. Closed flower gall caused by *Contarinia nasturtii* on *Eruca sativa*.
- Fig. 4. Galls caused by *Contarinia nasturtii* on the inflorescence, leaf axils, leaves and veins of *Sisymbrium officinale* (hedge mustard).
- Fig. 5. Inflorescence and leaf axil gall of *Contarinia nasturtii* on *Brassica sinapistrum* (charlock).
- Fig. 6. Leaf galls of *Contarinia nasturtii* on *Isatis tinctoria* (woad).
- Fig. 7. Seed head of *Sisymbrium officinale* after an inflorescence gall caused by *Contarinia nasturtii*.
- Fig. 8. Inflorescence gall caused by *C. nasturtii* on *Lepidium sativum* (common cress).
- Fig. 9. Seed head of *Lepidium sativum* after an inflorescence gall caused by *Contarinia nasturtii*.

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OBSERVATIONS ON THE OCCURRENCE OF THE EELWORM, *PRATYLENCHUS PRATENSIS* FILIPJEV, IN DELPHINIUM ROOTS

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(With Plate 7 and 1 Text-figure)

Pratylenchus pratensis has been found associated with lesions on delphinium roots: its presence in the tissues is best demonstrated by a modification of the Flemming staining method.

At specific sites, the severity of delphinium root-rot was correlated with the soil population of *P. pratensis*. After infested soils had been fumigated with methyl bromide, delphiniums grown in the treated soils had more vigorous root systems, containing fewer eelworms per unit weight than those grown in untreated soil. The use of D-D also shows promise as a control measure against *P. pratensis*.

No pathogenic fungus has been found in the delphinium root lesions.

INTRODUCTION

Pratylenchus pratensis Filipjev is the common root lesion nematode in many parts of the world and has been widely recorded in the roots of a variety of crops, particularly in the U.S.A. In the British Isles, however, although it is known to be widely distributed in the soil, it has rarely been found to be of economic importance.

Since the early 1920's certain growers in the West Country have been experiencing difficulty in growing delphiniums on particular plots of ground, and eelworms were first seen in the root cortex of affected plants by Mrs Justham, then in the Plant Pathology Department, N.A.A.S., Bristol. Miss Britton, lately of the Entomology Department, identified the eelworms as *P. pratensis* and this was confirmed by Dr J. B. Goodey of Rothamsted in September 1949.

Delphiniums are propagated by stem cuttings, struck in sterile soil under glass, and transplanted out of doors in the spring. After one year the root-stocks are used for sale; after two years the main crop of flowers is produced. It was found that plants grown on in pots invariably produced a normal bushy root system which remained white and free from lesions. Plants transferred to certain outside plots of ground also rooted well; but in the winter months when growth is retarded, they rapidly developed root-rots. After a year, affected plants, apart from their poor vegetative growth, could be detected by their lack of grip on the soil when being lifted. Examination showed that the bulk of the fine roots had disappeared. Delphinium growers have been able to associate these symptoms with specific plots of ground.

During 1949 delphiniums growing on ground associated with root-rots showed the following succession of symptoms. Black, slit-like lesions were first noted lying along the axis of the roots. Later these lesions widen and deepen, affecting an increasing proportion of the cortical tissue. After a year, it was not difficult to find plants on which the fine roots had almost completely rotted away, the remaining roots being predominantly blackened. A piece of root 2 mm. long and $1\frac{1}{2}$ mm. thick was found to contain thirty adults and larvae of *P. pratensis* and twenty-nine eggs. Further examinations showed that badly affected root systems contained a very high population of this eelworm. Tarjan (1949) found that $95.4 \pm 3.5\%$ of meadow nematodes emerged from boxwood roots by the Baermann funnel technique, but very few eelworms could be induced to emerge by this method from delphinium roots.

For the demonstration of the eelworms in delphinium root tissue best results were given by a modification of the Flemming method (Steiner, 1927; Godfrey, 1929). Tangential sections of cortical tissue surrounding primary lesions were crushed between two microscope slides, and left overnight in Flemming solution to enable the stain to penetrate sufficiently to reach the nematodes (see Pl. 7, fig. 1).

SOIL POPULATIONS

The following apparatus was devised for assessing soil populations of *P. pratensis*. A sieve is made by soldering a fine copper gauze (80 mesh to 1 in.) on to the base of a piece of 2 in. copper pipe, approximately $\frac{3}{4}$ in. long and placed in a glass funnel, fitted with rubber tubing and screw clip. A known weight of moist soil, standardized by passing through a 3 mm. sieve, is placed in it. Water is then poured into the funnel so as to cover the soil surface. Active soil eelworms move out of the soil mass, through the sieve, and fall by gravity to the base of the neck of the funnel, from where they can be collected in a small volume of water by opening the tap. Very few eelworms were found to emerge after 48 hr., and all the data given refer to eelworms recovered after this interval.

Assessment of eelworm populations in soil extracts is facilitated by the use of a counting slide made from thin microscope slides secured by Canada balsam (Text-fig. 1). The liquid to be examined, introduced with a suitable pipette, forms a rectangular block. There are no meniscus effects at each end of this block. At each side, this difficulty is obviated by withdrawing a little liquid and replacing by water.

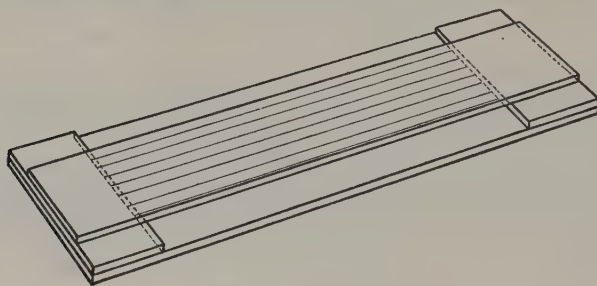
In 1949, counts were made by this method on soil samples placed in three categories by the growers for their effect on delphiniums (see Table 1).

A second series of samples, taken from areas indicated by the growers, was examined shortly afterwards. Here again, the growers' knowledge of the behaviour of each area of land corroborated the results of the eelworm counts. The results, and the percentage of organic matter present in each sample, are given in Table 2,

REPRODUCTION OF FIELD SYMPTOMS IN THE LABORATORY

No attempt has yet been made to produce a pure culture of *P. pratensis*. However, the production of lesions on delphinium roots in the laboratory, by means of a crude inoculum obtained by crushing badly affected roots, is thought worthy of a brief note. Potted plants grown in sterile soil, in spring 1949, were treated in two ways:

(a) The roots of an undamaged plant were washed free of soil, and 1 in. lengths of four roots were sleeved by small glass tubes, plugged at one end with compacted sterile soil. The tubes were then filled with sterile sand. Into two of the tubes a



Text-fig. 1. Diagram of counting slide.

TABLE 1. *Eelworm populations extracted from 5 g. moist soil*

Grower's description ...	'Good' area	'Moderate' area	'Bad' area
Population of <i>P. pratensis</i>	3	8	19
Total eelworm population	156	150	153

TABLE 2. *Association of grower's description of site with population of Pratylenchus pratensis extracted from 5 g. soil samples*

Grower's description ...	'Clean land'	'Fairly good'	'Moderate'	'Bad'
Population of <i>P. pratensis</i>	1	3	6	22
Total eelworm population	42	91	100	208
Percentage organic matter	4.64	5.82	6.16	7.00

small quantity of inoculum from infested roots was introduced, the remaining two received water. The plant bearing the sleeved roots was then repotted in its original soil.

(b) A small quantity of inoculum was poured into holes made in the soil surface of two other pots in which delphinium plants were growing; two further plants received water.

In the autumn of 1949 the plants given treatment (a) showed well-developed lesions on the inoculated roots. The plants inoculated in treatment (b) had a number of slit-like primary lesions on their roots. *P. pratensis* was readily found in association with all the lesions. No lesions could be found on the uninoculated controls.

SOIL TREATMENT

In the spring of 1949 a small pilot trial was carried out on a piece of ground known to be heavily infested with *P. pratensis*. After thorough mixing of the soil, eight plots, 1 yard square, were enclosed by corrugated asbestos sheets sunk 1 ft. into the ground. The treatments were: (1) methyl bromide, (2) chlorophenol, (3) cresylic acid, (4) control, each duplicated.

The plots to be fumigated with methyl bromide were given a water seal and covered with balloon fabric, the edges being buried in wet soil. A capsule containing 20 c.c. methyl bromide was buried 6 in. deep in the centre of each plot. The capsule was broken by a blow from an iron bar with a chisel edge passed through a hole in the fabric. The bar was then quickly removed and the hole sealed by a sticky flap. The fabric was removed from each plot after 3 days.

The chlorophenol was used at $\frac{1}{2}\%$ strength and watered on at 2 and 4 gall. per plot respectively. Horticultural cresylic acid ($2\frac{1}{2}\%$) was used for the third treatment at the same rates. (It has since been found that these quantities were insufficient to wet the soil to the required depth.)

The above treatments were applied on 9 May, and on 27 May each plot was planted up with eight strongly-rooted delphinium cuttings, struck in sterile soil. Two varieties were used, C. H. Middleton, a vigorous variety, and Wild Wales, much less vigorous.

In December 1949 two plants (var. Wild Wales) were lifted at random from each plot. Plants from each untreated plot had sparse and blackened root systems; those from each plot treated with methyl bromide were white and bushy. Roots from the other treatments were intermediate in appearance.

The remaining plants from all plots were lifted in March 1950. By this time the plants from each untreated plot had lost a large part of their root systems. Lesions were present on all the roots lifted, but these were few and primary on the roots lifted from soil treated with methyl bromide; a bushy, vigorous root system remained. As expected, the root-decay symptoms were more advanced on the less vigorous variety Wild Wales; variety C. H. Middleton had withstood attack more successfully. For this reason, the remaining four plants of the latter variety from each untreated and fumigated plot were used in an attempt to assess the populations of *P. pratensis* within their roots. Typical plants from untreated and fumigated soil are shown in Pl. 7, fig. 2.

After washing and the removal of surface water, the roots from the plants from each treatment were chopped into half-inch lengths. Representative 5 g. samples were crushed to a pulp and washed through a metal sieve (20 meshes to 1 in.). The fluid obtained from each sample was bottled, with the addition of formalin, when the macerated tissue soon settled, and the bulk of the liquid was decanted off. The remaining fluid was then made up to 100 c.c. and eelworm counts were carried out, as previously described, on aliquot samples of the agitated suspension (see Table 3).

The plant roots from untreated plots had a population of *P. pratensis* estimated to average 287 per g. of root, compared with 40 per g. in the roots grown in fumigated soil. It is likely that this difference in the growing plants was much greater, since many of the most heavily infested roots from the untreated plots were lost in lifting, and the difference in the size of the root systems (Pl. 7, fig. 2) meant that a much greater bulk of root was examined from the plants grown in fumigated soil.

USE OF D-D*

Demonstration plots, using D-D as the soil fumigant, were laid down in 1950. Owing to the laborious nature of the assessment of the results, and because of other practical difficulties, a replicated layout was not attempted. An area of ground approximately 50 ft. square, and known to be associated with severe root-rots, was kindly made available by growers.

TABLE 3. *Counts of Pratylenchus pratensis extracted from root samples, from small pilot trial*

Sample ...	Untreated soil			Soil fumigated with methyl bromide		
	A	B	C	A	B	C
Populations in 1 c.c. aliquots from 100 c.c. suspension	11	10	16	2	1	3
	13	7	19	1	2	2
	13	16	14	4	—	1
	10	14	21	1	3	3
	17	11	23	3	2	2
Totals	64	58	93	11	8	11
Treatment totals	215			30		
Av. population per g. root	287			40		

This area was thoroughly tilled and divided into five strips. D-D, used at 800 and 400 lb./acre was injected into the second and fourth strips respectively, strips 1, 3 and 5 being left untreated. The injections were carried out in March 1950 and the treated plots were given a water seal. The whole area was planted with vigorous rooted delphinium cuttings in May 1950.

Plant samples from each plot were taken in March 1951, when the areas treated with D-D could be readily picked out by the greater vegetative growth of the plants growing on them. Ten plants (five from each diagonal, taken at random) were lifted per plot, and used for the subsequent assessment of eelworm populations. The roots lifted from the treated plots were much bulkier than the rest; of the latter, the roots from strip 5 appeared least affected. Typical plants from strip 3 (untreated) and strip 4 (D-D 400 lb./acre) are shown in Pl. 7, fig. 3.

Chemical and physical methods of root maceration were not found to be satisfactory.

* A mixture of dichloropropane and dichloropropylene.

The roots were allowed to become air-dry and duplicate 1 g. samples from each plot were soaked in water and treated as already described. The results are set out in Table 4.

The figures have been submitted to a uniformity test. The significant lack of uniformity between duplicates ($P=2.6\%$) arises mainly from plot 1. This variation is, however, slight compared to that produced when plot totals are considered ($P<0.0002$). On the average, the roots lifted from untreated plots had an estimated population of 1097 *P. pratensis* present per g. of dried root, compared to 250 per g. in the roots lifted from ground treated with D-D. This method of control, although by no means complete, showed promise; conditions were not favourable to the efficiency of this material, since the soil was heavy, and the organic content was high (5.8%).

TABLE 4. *Counts of Pratylenchus pratensis extracted from root samples obtained from demonstration plots*

Strip ...	1		2		3		4		5	
Treatment ...	Control		D-D (800)		Control		D-D (400)		Control	
Sample ...	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
No. of <i>P. pratensis</i> found in	18	11	4	6	12	11	3	2	3	9
1 c.c. aliquots from 100 c.c.	15	10	1	3	12	13	3	2	10	10
suspension	18	7	3	5	9	10	2	—	9	5
	15	12	2	2	12	11	2	—	5	13
	16	10	3	4	16	9	1	2	8	10
Totals	82	50	13	20	61	54	11	6	35	47
χ^2	7.75		1.48		0.425		1.47		1.75	
Sum of $\chi^2=12.87$ D.F. = 5 $P=2.6\%$										
For plot totals, $\chi^2=132$ D.F. = 4 $P<0.0002$										

From such limited information, conclusions could only be tentative; however, it appeared, from the figures obtained from the control plots, that there had been an almost linear drift of infestation across the plots. The higher dosage rate of D-D, although injected into what was probably a higher initial population in plot 2, had dealt with it less successfully than the lower dosage rate in plot 4. It seemed probable that, under these conditions, two successive injections of D-D at 400 lb./acre might offer a better prospect of control than one injection at 800 lb./acre.

FIELD-SCALE TRIAL

A piece of ground of approximately $\frac{1}{4}$ acre, known to be associated with root-rot of delphiniums, was 'rototilled' and injected twice with D-D at 400 lb./acre during the summer of 1952 in order to prepare it for delphiniums in 1953. An attempt has been made to trace infestation of *P. pratensis* in this plot at different stages during the treatment. Fifty soil samples were taken to a depth of 6 in. over the area on three dates. From the bulked sample, the populations present were estimated from

four 10 g. replicates, using the technique already described; the soils having approximately 20% moisture content. A small refinement was used to provide a clear soil extract. The gauze mesh on the sieve of each unit was covered with a disk of bolting silk, having 20,000 pores to the square inch, each pore being approximately 0.005 by 0.0025 in. in size.

The ground was injected with D-D on the 27 June, and again in early September, and the data obtained from soil extracts are given in Table 5.

TABLE 5. *Nematode populations extracted from soil samples, from ground injected twice with D-D*

Sample ...	Pre-injection (25. iv. 52)		After first injection (26. viii. 52)		After second injection (10. x. 52)	
	<i>P. pratensis</i>	Others	<i>P. pratensis</i>	Others	<i>P. pratensis</i>	Others
Populations	14	103	1	17	—	18
extracted from	9	108	1	12	—	14
four 10 g.	12	109	2	14	—	20
replicates	8	97	1	16	1	15
Totals	43	417	5	59	1	67

DISCUSSION

P. pratensis is often found in plant roots associated with other organisms. Hastings & Bosher (1938) have studied the relationship of this nematode with the fungus *Cylindrocarpon* in a variety of hosts. Although fungi have been isolated from diseased delphinium roots, their pathogenicity has never been established. No other parasitic eelworms have been found by the writer in diseased root tissue; a very few specimens, thought to be of the genus *Paratylenchus*, have been found in soil and root extracts.

Experience in the West Country has shown that ground associated with this type of root-rot is not cleaned by rotation. Severe symptoms have been produced after delphiniums have been absent from the area for as long as 12 years. It is possible that a number of herbaceous plants are used as hosts by this eelworm, but it has not yet been found associated with root damage to any other cultivated plant. Weed hosts are not considered important, since the standard of hygiene on the nurseries visited is so high. Reddish lesions have, however, been found on the roots of *Senecio vulgaris*, with *Pratylenchus pratensis* present.

Allen and Raski (1952) have recorded a *Pratylenchus* species associated with extensive root-rots in tuberous begonia in California. This plant is grown extensively on the nurseries visited during the course of this work, but no damage has been seen or reported.

The author is grateful to Mr L. N. Staniland for his ready help and co-operation, and to Mr R. Sleigh, who rendered valuable assistance in the early stages of the work. Mr J. G. Mayor was responsible for all the photographs.

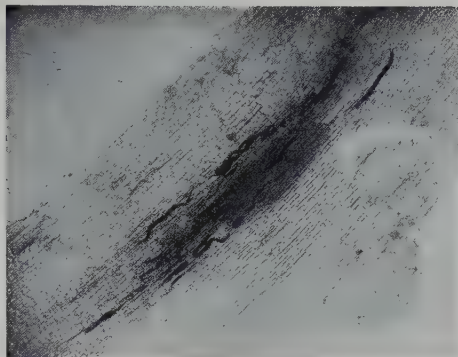


Fig. 1.



Fig. 2a.



Fig. 2b.



Fig. 3a.



Fig. 3b.

TONE—*Eelworm* *Pratylenchus pratensis* in *delphinium* roots

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EXPLANATION OF PLATE 7

- Fig. 1. Photomicrograph of primary lesion on delphinium root, stained in Flemming solution.
- Fig. 2. Delphinium roots from small pilot trial (see text). (a) Typical roots from untreated soil. (b) Typical roots from soil treated with methyl bromide.
- Fig. 3. Delphinium roots from demonstration plots. (a) Typical roots from untreated plot (plot 3). (b) Typical roots from plot treated with D-D at 400 lbs/acre (plot 4).

(Received 19 January 1953)

THE EFFECTS OF VARYING THE WATER SUPPLY OF PLANTS ON THEIR SUSCEPTIBILITY TO INFECTION WITH VIRUSES

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(With Plate 8)

Increasing the amount of water supplied to plants before they were inoculated with viruses greatly increased their susceptibility to infection; plants that received unlimited water produced 10 or more times as many local lesions as plants that received only enough to prevent wilting. Susceptibility was increased throughout the year, but the full response occurred in 2 weeks in winter and 4 weeks in summer. Plants that received unlimited water for the 2 weeks immediately preceding inoculation were no more susceptible than those that received it during the previous 2 weeks, although the external appearance of the plants differed at the time of inoculation. Varying water supply after inoculation did not affect the numbers of lesions.

The differences in susceptibility to infection produced by differential watering were decreased, but not abolished, by growing plants under shade or by incorporating a diatomaceous earth in the inoculum.

Increasing water produced plants with larger and more succulent leaves; the cuticular layer was thinner, and the palisade tissue was less regularly arranged than in the plants kept dry. The increased susceptibility caused by an abundant water supply may be at least partly due to these structural differences, which allow the leaf to be damaged more easily when rubbed with inocula and so present more entry points for virus particles.

There are several references in the literature to effects of varying soil moisture on the incidence of virus diseases, but most are observations made in conditions that do not allow the factors involved to be critically evaluated. In crops, not only are variations in soil moisture correlated with other climatic changes, but it is also difficult to decide whether differences arise because the supply of water directly affects the ease with which a plant contracts infection, or to some other effect, such as the extent to which infected plants produced clearly recognizable symptoms or the extent to which the activities of some insect or other vector have been influenced. The experiments described in the present paper were done with sap-transmissible viruses to find how variations in the amount of water supplied to plants affect their susceptibility to infection, as indicated by the numbers of local lesions produced. The results show that increasing the water supply, from a level that permits growth and just prevents wilting, to the maximum a plant can use, greatly increases the ease with which infection occurs.

MATERIALS AND METHODS

The viruses and hosts used were tobacco mosaic virus in *Nicotiana glutinosa* L. and tobacco *N. tabacum* L. var. White Burley; tomato aucuba mosaic virus in tobacco; tomato bushy stunt virus in *N. glutinosa*; potato virus X in tobacco and potato virus Y in tobacco.

The experiments were done in glasshouses that were heated during the winter. Plants were grown in pots containing 1 kg. of soil and were selected for uniformity (at the four-leaf stage) from large batches of seedlings. At least five, and usually ten, plants were used for each treatment. The positions occupied on the glasshouse benches by plants that were watered differently, were randomized to reduce errors arising from positional effects.

After preliminary tests, in which various amounts of water were given to plants in different ways, the procedure was standardized to give three levels, which will be called wet, medium and dry. The two extremes were achieved respectively by (1) standing the pots in saucers that always contained water, so that the soil was continually wet, and (2) by pouring just enough water on to the soil to prevent the plants from wilting; the amount needed for this varied between 5 and 20 ml. per day, depending on the season and the size of the plants. The plants in the medium treatment received 5 times as much water as those in the dry treatment; this is rather less than the amount given in normal glasshouse practice.

Tobacco mosaic virus was used as purified preparation and the inoculum usually contained 10 mg./l.; inocula for other viruses was freshly expressed sap from infected plants diluted 1/10 with water. Three leaves on each tobacco plant and four leaves on each *N. glutinosa* plant were inoculated by rubbing their upper surfaces, as uniformly as possible, with the forefinger wet with inoculum. The leaves were washed with running water immediately afterwards to remove excess inoculum.

At the time of inoculation the leaves were measured and their areas calculated, using the factors given by Sadasivan (1940). As the size of leaf varied widely within the treatments, the lesion counts were expressed as number per unit area (100 sq.cm.), in addition to the more customary method of number per whole or half leaf. For statistical analyses, the numbers of lesions on each leaf were transformed according to the formula $y = \log_{10}(x + c)$ (Kleczkowski, 1949), where x is the number of lesions per unit area of each leaf and c is a constant whose value was assessed as 10.

EFFECTS ON APPEARANCE AND CONSTITUTION OF PLANTS

The watering treatments considerably affected both the appearance (Pl. 8, fig. 1) and constitution of the plants. The leaves of plants kept wet for a month were larger, more fragile and lighter green than leaves of plants kept dry, which were small, tough and very dark green. Plants receiving a medium water supply had leaves which were intermediate in size and colour, but their texture approximated more

to that of the wet rather than to that of the dry treatments. Although the treatments affected the heights of the plants and the size of the leaves, they did not affect the number of leaves produced by the plants. The differences in leaf areas were considerable; the proportions in the dry, medium and wet treatments being about 1:2:3.

Several sets of equivalent leaves from plants that received the three treatments were compared for various properties. Table 1 shows two such comparisons made in May and January. The experiments ran for 4 weeks and then the plants were harvested and weighed. Thirty leaves were picked from ten plants of each treatment; they were weighed, minced in a meat mincer, the sap was expressed by squeezing through muslin, and its volume, nitrogen and carbohydrate contents were

TABLE 1. *Variations in the properties of healthy Nicotiana glutinosa plants caused by varying the water supply*

	Wet	Medium	Dry
(a) Ten plants per treatment. Sampled in May			
Mean leaf area (sq.cm.)	212	129	77
Mean height of plants (cm.)	14.0	9.9	9.1
Fresh wt. of plants (g.)	124	123	81
Dry wt. as % fresh wt. of leaves	8.9	8.1	8.6
Volume of sap in 100 g. leaf (ml.)	69	67	65
Total nitrogen in expressed sap (mg./ml.)	4.25	3.98	4.68
Total carbohydrate in expressed sap (mg./ml.)	71.5	20.0	21.0
(b) Ten plants per treatment. Sampled in January			
Mean leaf area (sq.cm.)	73	63	28
Mean height of plants (cm.)	11.0	8.8	7.0
Fresh wt. of plants (g.)	126	128	96
Dry wt. as % fresh wt. of leaves	7.0	7.0	9.0
Volume of sap in 100 g. leaf (ml.)	58	72	60
Total carbohydrate in sap (mg./ml.)	3.80	3.75	4.00

measured. At both times of the year, differential watering affected growth, but the large effect on carbohydrate content of sap produced in May was not obtained in January when conditions for photosynthesis were bad. The nitrogen content of sap was not measured for the January samples, but it is unlikely that it differed much with the different treatments, for the total nitrogen was 7.0% of the dry weight of the plants kept dry, 7.8% for the medium, and 7.4 for the wet plants. There were differences in the phosphorus and potassium contents. The dry plants had 0.7 and 5.5% of their dry weight as phosphorus and potassium, the medium plants 0.9 and 7.7% and the wet plants 1.1 and 8.5%.

EFFECTS ON NUMBERS OF LOCAL LESIONS

Table 2 gives the results of experiments with four different viruses and two host plants and shows that, with all, increasing the amount of water supplied to plants before inoculation increases the numbers of local lesions produced both per leaf and per unit area of leaf. The results of ten further experiments made at different times of the year with *N. glutinosa* are recorded in Table 3. They show that the number

of lesions produced, by both tobacco mosaic and tomato bushy stunt viruses, was greater in heavily watered than in lightly watered plants, despite the fact that, in different experiments, the plants were growing under widely differing conditions of day length and light intensity. No differences were noticed between the type of lesion produced by infection in plants that had received different amounts of water (Pl. 8, fig. 2). Nor was the normal gradient of susceptibility in *N. glutinosa* plants to the two viruses affected; at all three levels of watering the upper leaves produced more lesions than the lower ones with tomato bushy stunt virus, and fewer than the lower ones with tobacco mosaic virus.

TABLE 2. *The effects of variations in water supply on susceptibility to infection with different viruses*

	Wet	Medium	Dry	S.E.	M.S.D.*
(a) Potato virus X in tobacco (September)					
Lesions per leaf	174.7	108.1	28.8	—	—
Lesions per 100 sq.cm.	101.5	79.7	35.2	—	—
Transformed values per 100 sq.cm.†	1.92	1.90	1.61	±0.116	0.35
(b) Potato virus Y in tobacco (October)					
Lesions per leaf (starch-iodine lesions)	16.8	4.2	2.1	—	—
Lesions per 100 sq.cm.	10.7	4.3	2.9	—	—
Transformed values per 100 sq.cm.†	1.29	1.14	1.09	±0.041	0.12
(c) Tobacco mosaic virus in <i>N. glutinosa</i> (August)					
Lesions per ½ leaf	28.1	3.7	1.4	—	—
Lesions per 100 sq.cm.	120.3	17.8	9.9	—	—
Transformed values per 100 sq.cm.†	1.91	1.31	1.24	±0.067	0.20
(d) Tomato bushy stunt virus in <i>N. glutinosa</i> (May)					
Lesions per ½ leaf	28.9	3.8	1.5	—	—
Lesions per 100 sq.cm.	135.7	24.6	10.2	—	—
Transformed values per 100 sq.cm.†	1.92	1.40	1.24	±0.084	0.25

* M.S.D. = the difference statistically significant at $P=0.05$.

† Transformation was the mean of $\log_{10}(x+10)$, where x was the number of lesions per 100 sq.cm.

The sap of many species of plants contains materials that, if mixed with viruses *in vitro*, prevent infection when the mixtures are inoculated to susceptible plants. There is no evidence that these materials have any such inhibitory action *in vivo*, but one possible explanation for variations in susceptibility to infection by varying watering would be that plants kept dry contain more of such inhibiting substances. No evidence was found to support this idea, and if it is correct then the substances are not liberated in sap squeezed from macerated leaves. When sap from *N. glutinosa* leaves was mixed with purified tobacco mosaic virus and inoculated to healthy *N. glutinosa* leaves, fewer lesions were produced than by comparable solutions of virus in water, but sap from leaves of plants that had received the three different levels of watering reduced the numbers to the same extent.

TABLE 3. *The effects of water supply on the susceptibility of Nicotiana glutinosa plants to infection*

	Tobacco mosaic virus					Tomato bushy stunt virus				
	Wet	Medium	Dry	S.E.	M.S.D.	Wet	Medium	Dry	S.E.	M.S.D.
February										
Lesions per $\frac{1}{2}$ leaf	8.4	7.1	2.9	—	—	22.2	16.8	6.9	—	—
Lesions per 100 sq.cm.	59.6	50.3	32.3	—	—	160.2	116.1	80.3	—	—
Transformed values per 100sq.cm.*	1.71	1.70	1.49	± 0.076	0.23	2.15	2.03	1.85	± 0.065	0.21
April										
Lesions per $\frac{1}{2}$ leaf	44.8	22.9	8.3	—	—	48.8	10.8	2.6	—	—
Lesions per 100 sq.cm.	128.0	50.3	25.5	—	—	131.3	23.1	10.9	—	—
Transformed values per 100sq.cm.*	2.14	1.78	1.55	± 0.076	0.23	2.15	1.52	1.32	± 0.048	0.18
May										
Lesions per $\frac{1}{2}$ leaf	18.3	4.5	4.3	—	—	3.8	0.6	0.9	—	—
Lesions per 100 sq.cm.	53.5	19.2	22.4	—	—	10.7	2.7	5.1	—	—
Transformed values per 100sq.cm.*	1.71	1.38	1.42	± 0.049	0.14	1.25	1.09	1.14	± 0.036	0.10
September										
Lesions per $\frac{1}{2}$ leaf	17.9	8.3	1.9	—	—	113.2	46.8	8.0	—	—
Lesions per 100 sq.cm.	42.2	25.3	11.1	—	—	278.2	167.0	56.8	—	—
Transformed values per 100sq.cm.*	1.61	1.43	1.22	± 0.072	0.22	2.33	2.11	1.73	± 0.069	0.21
October										
Lesions per $\frac{1}{2}$ leaf	109.7	60.3	13.4	—	—	64.7	37.7	5.3	—	—
Lesions per 100 sq.cm.	326.4	194.1	55.7	—	—	204.8	127.6	22.8	—	—
Transformed values per 100sq.cm.*	2.46	2.19	1.67	± 0.067	0.19	2.19	1.98	1.41	± 0.063	0.18

10 plants per treatment in each experiment.

* Transformation was the mean of $\log_{10}(x+10)$ where x was the number of lesions per 100 sq.cm.TABLE 4. *The time necessary for differential watering to affect susceptibility*

	Tobacco mosaic virus					Tomato bushy stunt virus				
	Weeks					Weeks				
	1	2	4	S.E.	M.S.D.	1	2	4	S.E.	M.S.D.
	(a) November									
Wet										
Lesions per leaf	6.3	8.0	23.9	—	—	34.8	58.1	107.0	—	—
Lesions per 100 sq.cm.	26.5	43.5	105.9	—	—	156.8	316.1	459.4	—	—
Transformed values per 100 sq.cm.*	1.46	1.57	1.91	± 0.083	0.25	2.15	2.39	2.53	± 0.107	0.31
Dry										
Lesions per leaf	2.5	0.6	1.1	—	—	24.3	11.1	9.8	—	—
Lesions per 100 sq.cm.	11.6	5.1	13.7	—	—	126.3	82.3	94.0	—	—
Transformed values per 100sq.cm.*	1.28	1.12	1.21	± 0.083	0.25	2.08	1.67	1.69	± 0.107	0.31
Difference	0.18	0.45	0.70	± 0.117	0.23	0.07	0.72	0.83	± 0.152	0.40
	(b) July									
Wet										
Lesions per leaf	22.1	18.3	45.4	—	—					
Lesions per 100 sq.cm.	33.6	26.3	51.1	—	—					
Transformed values per 100sq.cm.*	1.58	1.52	1.74	± 0.063	0.19					
Dry										
Lesions per leaf	13.5	7.4	3.7	—	—					
Lesions per 100 sq.cm.	28.6	19.9	14.1	—	—					
Transformed values per 100sq.cm.*	1.57	1.44	1.34	± 0.063	0.19					
Difference	0.01	0.08	0.40	± 0.089	0.18					

* Transformation was the mean of $\log_{10}(x+10)$, where x was the number of lesions per 100 sq.cm.

For the experiments recorded in Tables 2 and 3, the plants were watered differentially for the 4 weeks before they were inoculated. This period was selected because it produced considerable differences in the size of plants and external appearance of the leaves. To see how soon differences in susceptibility were produced, plants were given the two extreme levels, wet and dry, for periods of 1, 2 and 4 weeks immediately before inoculation. The treatments were so timed that all plants were inoculated at the same time. Plants not due for differential watering were given 100 ml. of water per day until they came under treatment.

An experiment with *N. glutinosa* and tobacco mosaic and tomato bushy stunt viruses, done in November (Table 4a), gave significant differences between the number of lesions produced on the wet and dry plants after 2 weeks' treatment but not after 1; the difference in susceptibility between the two lots of plants was increased only a little when the period of differential watering was extended to 4 weeks. A comparable experiment with tobacco mosaic virus in July gave a different result (Table 4b). This time there was no significant increase in lesion numbers after

TABLE 5. *The effect of alternating periods of heavy and light watering on the susceptibility of Nicotiana glutinosa plants to infection with tobacco mosaic virus*

	Wet 4 weeks	Wet 2 weeks, dry 2 weeks	Dry 4 weeks	Dry 2 weeks, wet 2 weeks	S.E.	M.S.D.
Lesions per leaf	71.6	20.3	1.3	11.1	—	—
Lesions per 100 sq.cm.	84.9	32.4	4.2	29.4	—	—
Transformed values per 100 sq.cm.*	1.90	1.54	1.13	1.54	± 0.071	0.21

* Transformation was the mean of $\log_{10}(x+10)$, where x was the number of lesions per 100 sq. cm.

2 weeks of different watering, but there was after 4. Hence, although susceptibility to infection is increased by heavy watering at all times of the year, the effect seems to be obtained sooner in winter than in summer.

Table 5 gives the results of an experiment in which plants kept wet or dry for the 4 weeks before inoculation were compared with plants that were kept wet for either the first or second 2 weeks of the 4 and dry for the other 2 weeks. Susceptibility to infection was affected by the amount of water supplied in either 2-week period and there was no significant difference in the numbers of lesions produced per unit area of leaf on plants kept wet for the 2 weeks immediately before inoculation or for the preceding 2 weeks. This similarity in susceptibility of the two lots of plants would not have been suspected from their external appearance, for they looked very different at the time of inoculation. Heavy watering at the start of the experiment affected the rate of growth of the plants, and in the second 2 weeks affected the size, colour and texture of leaves. Plants kept wet for the first 2 weeks were almost as tall as those kept wet for all 4 weeks, but their leaves were smaller, harder and darker green. Plants kept dry for the first 2 weeks were only slightly taller than

those kept dry the whole time, but their leaves were larger, lighter green and less tough.

In all the experiments so far described, plants were watered uniformly after inoculation, to restrict any effects of differential watering to pre-inoculation treatments. Lesions were counted 5-7 days after plants were inoculated, and as more than a week of differential watering before inoculation is needed to produce any measurable effects on susceptibility, it was expected that the amount of water supplied to plants after they had been inoculated would not affect susceptibility. This expectation was realized by experiments in which batches of plants, kept either wet or dry for 4 weeks before inoculation, were each divided into two lots after inoculation, one of which was kept wet and the other dry, when the post-inoculation treatments consistently failed to affect the number of lesions produced.

THE INTERACTION OF WATER SUPPLY WITH OTHER FACTORS THAT INFLUENCE SUSCEPTIBILITY

Bawden & Roberts (1947, 1948) showed that plants are more easily infected by viruses when grown under reduced light intensity than when grown under ordinary glasshouse conditions. Shaded plants have fragile leaves, resembling those produced by plants given an unlimited supply of water. It was of interest to see whether the increased susceptibility produced from the two treatments arose from similar causes and so would cancel one another or whether they would be additive. Experiments were made in which plants given three levels of watering were divided into two lots, one of which was kept under ordinary glasshouse conditions for the month before inoculation and the other was kept under reduced light intensity (approximately one-third of the other) by enclosing them in a muslin-covered frame. At the time of inoculation, all the shaded plants had larger and more fragile leaves than their unshaded counterparts. After inoculation, the plants were kept in ordinary glasshouse conditions and watered uniformly. Table 6 shows the results of one such experiment with tobacco mosaic virus and one with tomato bushy stunt virus. It is clear that increasing the amount of water given to plants increased their susceptibility to infection by both viruses, whether the plants were shaded or not, but that unshaded plants were the more affected. Hence the effects of shading and watering seem partly but not wholly independent of one another.

Another thing that greatly increases the number of lesions produced by a given inoculum, is the addition of an abrasive such as fine carborundum or 'Celite' (a diatomaceous silica). Kalmus & Kassanis (1945) found that the addition of 'Celite' was equivalent to increasing the virus concentration of the inoculum by about a hundredfold and suggested it acted either by increasing the number of wounds or by exposing some cells otherwise not exposed to the virus by rubbing. Experiments were made to see how the susceptibility of plants that received different amounts of water were affected by incorporating an abrasive in the inoculum. Table 7 shows the result of an experiment in which plants were raised

as usual for a month with three different levels of water, but with the variation that the opposite halves of leaves were inoculated with and without 'Celite'. Half of each leaf was first rubbed with a solution containing 10 mg. tobacco mosaic virus per litre, and then the other half was lightly dusted with 'Celite' and rubbed with the same inoculum. 'Celite' significantly increased the numbers of lesions produced by all three lots of plants, but its effect on number of lesions produced was greater with plants kept dry than with those kept wet. The comparative lack of

TABLE 6. *The effects of variations in water supply and shading on susceptibility to infection*

	Wet	Medium	Dry	S.E.	M.S.D.
(a) Tobacco mosaic virus in <i>N. glutinosa</i> (August)					
With shade					
Lesions per $\frac{1}{2}$ leaf	9.6	2.4	1.1	—	—
Lesions per 100 sq.cm.	30.7	10.6	9.1	—	—
Transformed values per 100 sq.cm.*	1.54	1.25	1.21	± 0.044	0.13
Without shade					
Lesions per $\frac{1}{2}$ leaf	4.7	0.5	0.1	—	—
Lesions per 100 sq.cm.	21.6	4.7	0.9	—	—
Transformed values per 100 sq.cm.*	1.45	1.11	1.02	± 0.044	0.13
Response to shade	0.09	0.14†	0.19†	± 0.064	0.13
(b) Tomato bushy stunt virus in <i>N. glutinosa</i> (August)					
With shade					
Lesions per $\frac{1}{2}$ leaf	47.3	4.8	4.2	—	—
Lesions per 100 sq.cm.	155.3	23.8	37.0	—	—
Transformed values per 100 sq.cm.*	1.93	1.41	1.50	—	—
Without shade					
Lesions per $\frac{1}{2}$ leaf	10.3	1.5	0.2	—	—
Lesions per 100 sq.cm.	54.1	12.5	2.1	—	—
Transformed values per 100 sq.cm.	1.59	1.28	1.05	± 0.069	0.19
Response to shade	0.34†	0.13	0.45†	± 0.104	0.21

* Transformation was the mean of $\log_{10}(x+10)$, where x was the number of lesions per 100 sq.cm.

† Significant responses.

effect of 'Celite' on plants supplied with unlimited water was confirmed in other experiments in which plants that received different amounts of water were inoculated with tobacco mosaic virus at a range of concentrations, opposite half-leaves being rubbed with and without 'Celite'. Table 8 gives the results of one such experiment; responses to 'Celite' are given as the differences between transformed numbers of lesions per unit area of leaf produced on half-leaves inoculated with and without 'Celite', and the only statistically significant response with plants kept wet was with the most concentrated inoculum. It is of some interest, though the fact has no known interpretation, that on all plants the response to 'Celite' increased as the concentration of the inoculum increased.

WATER SUPPLY AND LEAF STRUCTURE

There are many references in the literature describing changes in the anatomy of leaves caused by varying water supply, but none deals with species used in my experiments or with plants grown in comparable conditions. The difference in external appearance of leaves of *N. glutinosa* supplied with different amounts of water suggested that their internal structure would also differ, a suggestion that the examination of sections substantiated. Considerable difficulty was met in

TABLE 7. *The influence of an abrasive on the susceptibility of differentially watered plants. Tobacco mosaic virus in Nicotiana glutinosa (May)*

	Wet	Medium	Dry	S.E.	M.S.D.
With 'Celite'					
Lesions per $\frac{1}{2}$ leaf	71.5	62.9	35.8	—	—
Lesions per 100 sq.cm.	241.5	233.2	142.7	—	—
Transformed values per 100 sq.cm.*	2.36	2.34	2.08	± 0.088	0.26
Without 'Celite'					
Lesions per $\frac{1}{2}$ leaf	45.8	8.7	4.9	—	—
Lesions per 100 sq.cm.	136.1	32.4	18.6	—	—
Transformed values per 100 sq.cm.*	2.03	1.57	1.39	± 0.088	0.26
Response	0.33†	0.77†	0.69†	± 0.078	0.16

* Transformation was the mean of $\log_{10}(x+10)$, where x was the number of lesions per 100 sq.cm.

† Significant responses.

TABLE 8. *The responses to 'Celite' of differently watered plants when inoculated with tobacco mosaic virus at various concentration*

Concentration of inoculum (mg./l.)	Wet	Medium	Dry	S.E.	M.S.D.
100	0.32*	0.33*	0.69*	—	—
10	0.08	0.27*	0.52*	—	—
1	—0.03	0.25*	0.20*	—	—
0.1	0.03	0.09	0.27*	—	—
0.01	0.10	—0.07	0.00	± 0.054	0.16

* Significant responses.

finding a suitable method for fixing and staining sections of *N. glutinosa* leaves, and of many tried the following proved best. Small pieces of leaf were cut from leaves of comparable age on plants that had been kept wet or dry for the previous 4 weeks. These were dipped in a dilute solution of a detergent (commercial 'Teepol' at 1/1000 in water), transferred to tubes containing Craff 1 fixative, and then kept under vacuum until the pieces sank. The pieces of leaf were dehydrated, embedded in wax, sectioned and stained for 5-6 days with a 4% alcoholic solution of gossypimine (Lee & Priestley, 1924).

Figs. 3 and 4 (Pl. 8) are sections through comparable leaves of plants kept wet and dry, and show that considerable differences occur in all the layers of cells. The

leaves from plants given abundant water have a thinner cuticle than leaves from plants kept dry, but the cells in the epidermis, palisade and spongy parenchyma are all larger and more loosely packed, and the leaf itself is thicker. The greater compactness and regularity of cells in leaves from *N. glutinosa* plants kept dry agree with results described for other species (Clements, 1905; Maximov, 1929).

These differences in leaf structure provide the most plausible explanation for the effects that differential watering has on susceptibility to infection by mechanically inoculated viruses. The thicker cuticle and greater compactness of the cells in leaves from plants kept dry would probably increase resistance to injury when rubbed, whereas the thin cuticle and loosely packed cells of leaves from well-watered plants would seem more likely to suffer injuries and be readily penetrated by virus particles. This mechanical interpretation of the effect of watering in increasing liability to infection is also supported by the results of experiments in which inoculations were made with 'Celite', which increased infection much more in plants kept dry than in plants kept wet. Such a differential effect is to be expected if, as seems likely, the main effect of 'Celite' is to increase the number of wounds that are suitable points of entry for viruses.

A change in the anatomy of leaves, of course, only reflects other changes in physiological activities of the cells. These changes may also play a direct part in influencing the susceptibility to infection with viruses. However, it seems unlikely that they are very important, as changes in susceptibility as a result of varying water supply occur relatively slowly and are not measurable until leaf structure has also become modified. In this respect, effects of increasing water supply resemble those of reducing the light intensity under which plants are grown. Bawden & Roberts (1947, 1948) suggested that the increased susceptibility to infection produced by shading plants might occur at least partly because of increased susceptibility to injury, but the more rapid increase in susceptibility occasioned by keeping plants in the dark seemed to occur without the leaves becoming more fragile. No doubt there are many interacting factors that influence the establishment and multiplication of virus particles; the mechanical resistance of leaves to injury may be only one such factor, but it seems an important one.

I am indebted to Mr J. H. A. Dunwoody for help with the statistical analysis of some of the results.

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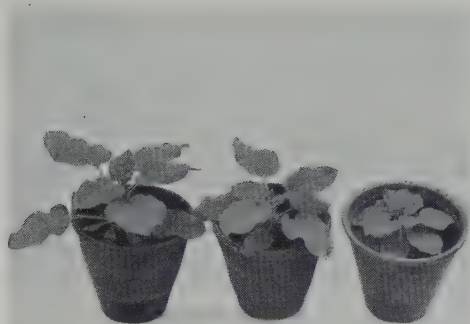
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EXPLANATION OF PLATE 8

- Fig. 1. The effect of three different levels of watering on the growth of *Nicotiana glutinosa* L. Plants photographed 1 month after start of treatment, left-hand plant wet, centre medium, right-hand dry.
- Fig. 2. Comparable leaves of *N. glutinosa* from plants kept wet (left) and dry showing the local lesions produced by tobacco mosaic virus.
- Fig. 3. Leaf section from *N. glutinosa* plant treated with an unlimited water supply. Fixed in CRAF 1 and stained with gossypimine. Note the weakly developed cuticle and disorganized palisade layer. Photographed with Wratten 'B' filter. $\times 225$.
- Fig. 4. Leaf section from *N. glutinosa* plant kept dry. Fixed in CRAF 1 and stained with gossypimine. Note the thick cuticle and the compact well-developed palisade cells. Photographed with Wratten 'B' filter. $\times 225$.

Figs. 1 and 2 were photographed by Mr V. Stansfield.

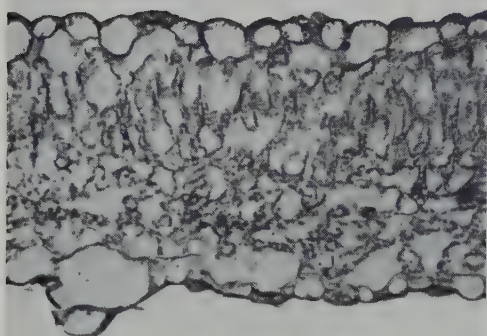
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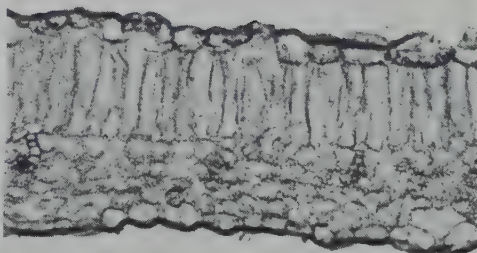
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TRACE-ELEMENT TOXICITIES IN OAT PLANTS

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Excessive amounts of nickel, cobalt, chromium, copper, zinc, manganese, molybdenum and aluminium in nutrient solutions supplied to oat plants in sand culture produce (a) chlorosis and (b) other symptoms specific to the element involved. The specific symptoms are distinct for each metal, although those of cobalt and nickel might be confused.

The toxic effects of nickel, cobalt, copper, zinc, manganese and molybdenum are associated with high concentrations of the element in the leaf tissue, but this is not always so with chromium and aluminium.

The toxic effects of nickel, chromium, copper and molybdenum are associated with a reduced nitrogen content of the plant. Nickel, cobalt, chromium, zinc and manganese increase the concentration of phosphorus in the tissue whilst aluminium decreases it, probably to a deficiency level.

Aluminium reduces the intensity of toxic symptoms produced by nickel—probably by reducing the uptake of nickel and phosphorus. Copper effectively reduces the leaf necrosis produced by nickel, but not the nickel content of the leaf tissue; it is suggested that one factor in nickel toxicity may be inhibition of one or more functions of copper. The other elements slightly increase chlorosis and some increase necrosis.

The order of activity of the elements in producing chlorosis is found to be $Ni > Cu > Co > Cr > Zn > Mo > Mn$. This order, which is related to that giving yield reduction and is similar to the order of stability of metal complexes, is discussed in relation to induced iron deficiency.

INTRODUCTION

Investigations on the toxicity associated with certain Aberdeenshire soils, developed on a mixed drift derived largely from ultrabasic igneous rocks in which serpentine predominates, showed that the soils, and plants grown on them, contained excessive concentrations of nickel (Hunter & Vergnano, 1952). Other trace elements (for example, cobalt, chromium and zinc) were also present in the acetic-acid extracts of the soils and in the plants in abnormally high concentrations (though less abnormal than nickel), but it was questionable if they were at toxic levels. The literature was of little help in interpreting the analytical figures obtained; although the data for acetic-soluble trace constituents of soil are often of considerable value, the interpretation of results is as yet empirical (Mitchell, 1948), and few figures are given in the literature for the composition of plants under toxic conditions. Further observations, made by the authors during preliminary investigations on the effect of nickel, indicated that the degree of nickel toxicity might be influenced by other

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trace elements; such a relationship was known to exist between nickel and the major-nutrient elements, and between nickel and iron (Crooke, Hunter & Vergnano, unpublished). The experiments described below were made to clarify the position.

The objects of the investigation were: (i) comparison of the toxicity symptoms produced in oat plants by nickel, cobalt, chromium, copper, zinc, manganese, molybdenum and aluminium; (ii) determination of the trace-element content of the leaves of plants affected slightly or seriously by excessive amounts of the individual trace elements; (iii) determination of the effect of individual trace elements on nickel toxicity. The application of the results to the determination of the trace-element status of ultrabasic soils and plants will be mentioned only briefly in this paper; it is intended to publish details at a later date.

EXPERIMENTAL

Oat plants (variety, Victory) were grown during the summer in acid-washed quartz sand (13% held by a 14-mesh and 99% by a 30-mesh British Standard sieve) in a bird-proof cage. The containers were 6 in. clay pots coated with bituminous paint, three pots being used for each treatment. Seeds were sown in the damp sand and were supplied with water until the shoots were $\frac{1}{4}$ in. long. Thereafter, adequate amounts of nutrient solutions were supplied twice daily, preceded by flushing with water. Water was given at other times if required.

The nutrient solutions were prepared by adding measured volumes of stock solutions to water and diluting with water to a predetermined volume. The composition per litre of the major-element stock solutions (and volumes required to prepare 10 l. of each nutrient solution) were: NaNO_3 , 250 g. (15 ml.); $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 50 g. (20 ml.); K_2SO_4 , 90 g. (20 ml.); $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 430 g. (15 ml.); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 250 g. (15 ml.). A basic level of trace elements was included in these solutions and was added as 10 ml. of a stock solution containing 6.40 g. ferric citrate, 0.98 g. $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.04 g. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.35 g. H_3BO_3 , per litre. The basic composition (p.p.m.) of the nutrient solutions was therefore: nitrogen, 138; phosphorus, 8.7; potassium, 81; calcium, 110; magnesium, 37; iron, 1.2; manganese, 0.24; copper, 0.01; boron, 0.06. Toxic concentrations of trace elements were supplied as components of the basic solution, being added as measured volumes of stock solutions along with the basic components; the concentrations of the trace elements used are given in the respective sections, nickel, cobalt, copper, zinc, manganese and aluminium being supplied as the sulphate, and chromium and molybdenum as potassium dichromate and sodium molybdate respectively. The pH of each solution was adjusted by sulphuric acid to 5.5, but a gradual increase to about 6.5 occurred when the solutions were in contact with the plant roots.

The plants were grown for 40 days after germination of the seed. At harvest, the average heights of plants were noted and selected treatments were sampled. The fully expanded (but not senescent) leaves were dried and the appropriate trace

elements (except aluminium) determined spectrochemically in the dry matter—an accuracy within $\pm 10\%$ being usually attained (Mitchell, 1948); aluminium was determined chemically. The middle thirds of stems (including leaf sheaths) were extracted in a blending instrument (Hester, 1940) with Morgan's reagent (Morgan, 1941), and nitrate, phosphate, potassium, calcium and magnesium were determined in the extracts; these figures were used as a measure of the nutrient status of the plants (Goodall & Gregory, 1947). Total nitrogen was also determined in the leaf dry matter of some samples, the results being given in Table 1. Unfortunately

TABLE 1. *Composition of stem extracts and other data*

Series	Concentration (p.p.m.) in nutrient solution		p.p.m. in stem extract				
		Ni	NO ₃	PO ₄	K	Ca	Mg
Nickel	—	0	116	23	273	16	14
	—	2.5	114	36	285	23	13
	—	5	101	42	262	22	17
	—	10	32	72	296	29	20
Total N (% in leaf dry matter); control=3.4, 10 p.p.m. Ni=2.1							
Cobalt	Co	Ni					
	0	—	116	23	273	16	14
	5	—	136	36	276	25	15
	15	—	155	87	257	28	17
	0	2.5	114	36	284	23	13
	2	2.5	99	60	257	27	15
Chromium	Cr	Ni					
	0	—	116	23	273	16	14
	10	—	198	33	284	22	13
	25	—	0	84	159	28	18
	0	2.5	114	36	284	23	13
	2	2.5	211	42	315	23	14
Total N (% in leaf dry matter); control=3.4, 25 p.p.m. Cr=1.8							
Copper	Cu	Ni					
	0	—	116	23	273	16	14
	5	—	112	9.0	276	28	16
	10	—	21	9.0	245	30	18
	20	—	0	23	237	20	16
	0	2.5	114	36	284	23	13
	5	2.5	32	14	312	36	21
	10	2.5	34	13	292	30	20
Total N (% in leaf dry matter); control=3.4, 20 p.p.m. Cu=2.6							
Zinc	Zn	Ni					
	0	—	116	23	273	16	14
	25	—	120	28	249	22	13
	100	—	92	72	284	25	15
	0	2.5	114	36	284	23	13
	25	2.5	101	39	273	23	12

TABLE I (*cont.*)

Series	Concentration (p.p.m.) in nutrient solution		p.p.m. in stem extract				
	Ni		NO ₃	PO ₄	K	Ca	Mg
Manganese	Mn	Ni					
	0	—	116	23	273	16	14
	150	—	168	36	284	14	11
	250	—	162	39	280	14	9.3
	0	2.5	114	36	284	23	13
	25	2.5	192	45	276	21	12
Molybdenum	Mo	Ni					
	0	—	116	23	273	16	14
	50	—	161	26	249	16	13
	200	—	20	23	261	17	14
	0	2.5	114	36	284	23	13
	2	2.5	161	42	296	24	14
	10	2.5	167	36	273	21	13
Total N (% in leaf dry matter); control = 3.4, 200 p.p.m. Mo = 2.2							
Aluminium	Al	Ni					
	0	—	116	23	273	16	14
	25	—	114	6	288	14	11
	100	—	88	4.8	249	8.8	6.5
	0	2.5	114	36	284	23	13
	25	2.5	167	8.4	249	21	12

only plants grown with critical levels of the trace elements could be analysed because of the amount of analytical work involved.

The recognized effects of excessive concentrations of trace elements on plants are the production of (*a*) chlorosis (due to induced iron deficiency) and (*b*) symptoms specific to the element involved (Gile, 1916; Wallace & Hewitt, 1946; Hewitt, 1946, 1947, 1948*a, b, c*; Millikan, 1947*a, b*, 1948; Morris & Pierre, 1949; Nicholas, 1950; Vergnano & Hunter, 1953). This distinction has been observed in the tables given below, in which the relative intensities of chlorosis and specific symptoms are recorded. Whenever chlorosis occurred some of the leaves affected were painted in the early stages with a 0.25% solution of ferrous sulphate containing 0.025% sodium lauryl sulphonate, and the response to the treatment always confirmed that iron deficiency was the cause of the chlorosis. The degree of symptoms is recorded in the tables according to the scale; nil, very low, low, moderate, high and very high; further differentiation is indicated by minus and plus signs.

Results

NICKEL

Concentration (p.p.m.) Ni in solution	Average height (cm.)	Degree of symptoms		Concentration (p.p.m.) Ni in leaf dry matter
		Chlorotic	Specific	
0	32	0	0	3.8
1	32	VL	VL	50
2.5	28	M	M	153
5	23	H	H	196
10	16	VH	VH	—
15	10	VH+	VH+	—
20	5	VH+	VH+	—
30	5	VH+	VH+	—

The leaves of all plants receiving nutrient solutions containing nickel developed chlorosis and necrosis, the former being diffuse and the latter being in the form of white longitudinal stripes. These symptoms have already been described in detail by the authors (Vergnano & Hunter, 1953).

The nickel content in the leaves of plants receiving nickel was considerable even where the level in the nutrient solution was low and at the 5 p.p.m. level, at which growth was first markedly reduced by nickel, the concentration of nickel in the leaves was very high.

The most obvious effects of nickel on the major-nutrient content of the stem extracts (see Table 1) were the increase in the phosphate content and, with high nickel concentrations in the solution, a decrease in nitrate.

Results

COBALT

Concentration (p.p.m.) in solution		Average height (cm.)	Degree of symptoms		Concentration (p.p.m.) in leaf dry matter	
Co	Ni		Chlorotic	Specific	Ni	Co
0	—	32	0	0	3.8	0.78
5	—	28	L	VL	—	116
10	—	26	M	L—	—	—
15	—	15	H	L+	—	640
50	—	10	o*	VH	—	—
100	—	5	o*	VH	—	—
Ni specific						
0	2.5	28	M	M	153	2.06
2	2.5	26	M+	M+	187	79
5	2.5	24	M+	M+	—	—
10	2.5	20	H	M+	—	—

* Masked by specific symptoms.

The symptoms typical of cobalt toxicity in oat plants are briefly, extensive interveinal chlorosis and less extensive white longitudinally-striped necrosis of the leaves. The symptoms of cobalt toxicity are therefore similar to those of nickel though they may be distinguished by (1) the chlorosis being prominently inter-

veinal (nickel usually diffuse), (2) the necrosis following the chlorotic pattern and thus giving narrower stripes than nickel, and (3) the necrosis developing more slowly than that of nickel and more slowly than the general toxicity, so that a low degree of necrosis may be associated with poor plants with narrow leaves.

Higher concentrations of cobalt than of nickel (either in solution or leaf) were required to produce the chlorotic and necrotic symptoms. For example, with 15 p.p.m. cobalt in the solution the degree of necrosis was still less than that produced by 2.5 p.p.m. nickel, though the cobalt concentration within the plant was high.

2 p.p.m. cobalt in the nutrient solution slightly intensified the effect of 2.5 p.p.m. nickel, and 79 p.p.m. cobalt were present in the leaf dry matter in addition to 187 p.p.m. nickel; once again, the level of cobalt in the plant leaves is considerably greater than normal before a cobalt effect is produced.

Though the specific symptoms of cobalt were not very marked at the 15 p.p.m. cobalt level in the solution, this level of cobalt was very toxic as shown by the reduction in height of plants.

The outstanding effect of excessive cobalt on the concentration of major elements in the tissue was the increase in phosphate content, an increase which was also found with excessive nickel.

Results

CHROMIUM

Concentration (p.p.m.) in solution		Average height (cm.)	Degree of symptoms		Concentration (p.p.m.) in leaf dry matter	
Cr	Ni		Chlorotic	Specific	Ni	Cr
0	—	32	0	0	3.8	0.4
5	—	30	VL	0	—	—
10	—	25	L	0	—	3.9
15	—	22	M	L	—	—
25	—	19	0*	H	—	252
50	—	13	0*	VH	—	—
Ni specific						
0	2.5	28	M	M	153	0.8
2	2.5	25	M+	M+	193	1.3
5	2.5	24	M+	M+	—	—
10	2.5	20	M+	M+	—	—

* Masked by specific symptoms.

Plants receiving 5 p.p.m. chromium in the nutrient solution were usually normal, but some showed slight diffuse chlorosis of the leaves. With 10 p.p.m. chromium, the plants were small and most of the leaves were slightly chlorotic. With 25 and 50 p.p.m. chromium, the plants were stunted; leaves were narrow and brownish red with small necrotic areas. Roots were normal with 5 and 10 p.p.m. chromium, and very poorly developed with the higher concentrations.

Chromium therefore produced specific symptoms of toxicity in oat plants quite

distinct from those of nickel and cobalt. Chromium further differed from nickel and cobalt in that, at the lowest effective level of chromium in the solution, relatively little chromium was found in the leaves, whilst at the highest toxic level, where symptoms were marked (25 p.p.m. chromium) the concentration of chromium was high.

2 p.p.m. chromium in the nutrient solution increased the degree of specific nickel symptoms and nickel uptake associated with 2.5 p.p.m. nickel in the solution, yet the leaf concentration of chromium was only 1.3 p.p.m. in the dry matter. Since the small increase in chromium concentration in the leaf with the less toxic levels in the solution seemed incompatible with the effect produced, it seemed probable at these levels that the principal chromium accumulation and action occurred in the roots, leaf symptoms being subsequent to root damage. Further investigation with several levels within the critical range is desirable.

The most striking effect of excessive chromium on the plant nutrient status was the low level of nitrogen in the plants with specific chromium symptoms; one effect of chromium was therefore the production of nitrogen deficiency. Another obvious change was the increase in the phosphate concentration in the tissue extract.

Results

COPPER

Concentration (p.p.m.) in solution		Average height (cm.)	Degree of symptoms		Concentration (p.p.m.) in leaf dry matter		
Cu	Ni		Chlorotic	Specific	Ni	Cu	
0	—	32	o	o	3·8	17	
2	—	32	VL	o	—	—	
5	—	30	M	o	—	—	
10	—	28	H	o	—	37	
20	—	18	o*	H	—	92	
25	—	18	o*	H+	—	—	
50	—	13	o*	VH	—	—	
75	—	9	o*	VH	—	—	
<div>Specific symptoms</div> <div> <div>Ni</div> <div>Cu</div> </div>							
0	2·5	28	M	M	o	153	22
2	2·5	28	M	M	o	—	—
5	2·5	26	M+	M—	o	122	24
10	2·5	28	H	VL	VL	160	40
20	2·5	15	o*	o	H	—	—

* Masked by specific symptoms.

Plants receiving 2 p.p.m. copper in the nutrient solution were usually normal, though some were slightly chlorotic (the chlorosis being interveinal); and those receiving 10 p.p.m. were very chlorotic. With concentrations of 20 p.p.m. copper and over, the plants were small and the leaves narrow, a few being chlorotic but the

majority were orange-coloured; the effect was greater with the higher concentrations. Roots were normal with 2 p.p.m. copper in the nutrient solution but small with higher concentrations.

The effect of copper on oat plants was therefore quite different from that of nickel and cobalt except that chlorosis was produced with low concentrations in the nutrient solution—although copper appeared to be slightly less effective in this respect than nickel. Specific symptoms of copper toxicity were also distinct from those of chromium, and the concentrations of copper in the solution which produced chlorosis were lower. The specific symptoms of copper toxicity (and marked yield reduction) did not appear until the concentration of copper in the solution was between 10 and 20 p.p.m.

The effect of 10 p.p.m. copper in the nutrient solution on the specific symptoms produced by 2.5 p.p.m. nickel was most marked. Necrotic symptoms were almost absent from the plants, although, as would be expected, the degree of chlorosis was greater. As the reduction in necrotic symptoms was not found to be paralleled by reduction in the nickel content of the leaves, it seems that evidence may be provided here of one effect of nickel toxicity, namely, the inhibition of copper function.

The main effect of copper on the major nutrient composition figures was a reduction in nitrogen status but a reduction in the phosphate level was also noticeable.

Results

ZINC

Concentration (p.p.m.) in solution		Average height (cm.)	Degree of symptoms		Concentration (p.p.m.) in leaf dry matter	
Zn	Ni		Chlorotic	Specific	Ni	Zn
0	—	32	o	o	3.8	140
10	—	30	o	o	—	—
25	—	29	VL	o	—	1700
50	—	27	M	o	—	—
75	—	28	M	o	—	—
100	—	16	H	H	—	7500
150	—	14	VH	VH	—	—
Ni specific						
0	2.5	28	M	M	153	240
2	2.5	28	M	M	—	—
5	2.5	27	M	M	—	—
10	2.5	28	M	M	—	—
25	2.5	24	M+	M	154	2000
50	2.5	22	M+	M	—	—

With 10 p.p.m. zinc in the nutrient solution, plants were normal and with 25 p.p.m. they were very slightly chlorotic (the chlorosis being diffuse). With 100 and 150 p.p.m. zinc, the plants were stunted and very chlorotic, and many leaf tips were yellow-red. Roots were normal when the concentrations of zinc were 75 p.p.m. or less, but were small with 100 and 150 p.p.m.

The toxic effects of zinc were therefore the production of chlorosis and, when the level of zinc in the nutrient solution was sufficiently high, specific symptoms which were unlike those of the trace elements already discussed; the levels at which these were produced were higher (both in solution and in the leaves) than for any of these other trace elements.

Relatively high concentrations of zinc in the nutrient solution affected only the chlorotic symptoms produced by 2.5 p.p.m. nickel, and as the nickel content of the leaves remained unchanged by the inclusion of zinc in the solution it is probable that the effect of zinc and nickel was additive. The vigour of the plants was seriously affected only by high concentrations of zinc (in solution, 100 p.p.m., and in the plant, 7500 p.p.m.).

Once again the main effect on the major-nutrient content was an increase in phosphate content.

Results

MANGANESE

Concentration (p.p.m.) in solution		Average height (cm.)	Degree of symptoms		Concentration (p.p.m.) in leaf dry matter	
Mn	Ni		Chlorotic	Specific	Ni	Mn
0	—	32	0	0	3.8	85
10	—	32	0	0	—	—
25	—	33	0	0	—	—
50	—	32	0	0	—	—
100	—	31	0	0	—	—
150	—	31	VL—	VL	—	3600
200	—	30	VL—	VL	—	—
250	—	31	VL	L	—	5400
500	—	20	L—	H	—	—
1000	—	18	M	VH	—	—
Ni specific						
0	2.5	28	M	M	153	95
5	2.5	28	M	M	—	—
10	2.5	27	M	M	—	—
25	2.5	24	M+	M	142	1970

Plants remained normal with solutions containing up to 100 p.p.m. manganese. With 150 and 200 p.p.m. manganese a slight degree of diffuse chlorosis was present in some of the leaves and a few of the leaf tips were red-coloured and necrotic; these symptoms were slightly more intense in the plants receiving 250 p.p.m. manganese. With 500 and 1000 p.p.m. manganese the plants were stunted and chlorotic (mottled), and the leaf tips were often red-brown and necrotic-necrosis occurring on other parts of the leaf also. With concentrations up to 100 p.p.m., the roots of the plants were normal; with higher concentrations they were dark brown and stunted. Manganese was even less active than zinc in producing chlorosis and specific symptoms, and the latter were different from those of the previously

discussed trace elements. High concentrations of manganese in the leaves were associated with manganese toxicity. Nickel chlorosis (but not necrosis) was increased slightly by excessive amounts of manganese in the nutrient solution, but the nickel content of the leaves was unaffected.

Analysis of the stem extracts indicated only small changes in major-nutrient content, including an increase in the phosphate content with toxic concentrations of manganese.

Results

MOLYBDENUM

Concentration (p.p.m.) in solution		Average height (cm.)	Degree of symptoms		Concentration (p.p.m.) in leaf dry matter	
Mo	Ni		Chlorotic	Specific	Ni	Mo
0	—	32	o	o	3·8	0·26
10	—	31	o	o	—	—
50	—	32	VL	o	—	480
100	—	30	L	L	—	—
200	—	24	M	L+	—	1540
Ni specific						
0	2·5	28	M	M	153	0·30
1	2·5	27	M	M	—	—
2	2·5	27	M	M	171	45
5	2·5	28	M	M	—	—
10	2·5	28	M+	M	118	340
25	2·5	25	M+	M	—	—

With concentrations up to 50 p.p.m. molybdenum in the solution, plants were normal, though at that level some showed a slight, diffused chlorosis. With 100 p.p.m. slight chlorosis was common and the leaf tips were often red-yellow. With 200 p.p.m. moderate chlorosis was present and red-yellow leaf tips were common. Roots were slightly smaller than normal with concentrations of molybdenum up to 100 p.p.m. and considerably smaller with 200 p.p.m.

The toxic effects of molybdenum were therefore specific and were associated, as were chlorosis and yield reduction, with high concentrations of molybdenum in the dry matter. The nickel content of the plants was not much altered by molybdenum, but the degree of chlorosis was slightly greater at the highest molybdenum level.

Molybdenum was considerably less effective in producing toxicity than the previous trace elements with the exception of manganese, which is best compared with molybdenum on a milli-equivalent basis at the 100 p.p.m. level. Only the highest level of molybdenum materially altered the major-nutrient status of the plants; the main change was a reduction in nitrogen status.

Results

ALUMINIUM

Concentration (p.p.m.) in solution		Average height (cm.)	Degree of symptoms		Concentration (p.p.m.) in leaf dry matter	
Al	Ni		Chlorotic	Specific	Ni	Al
0	—	32	o	o	3.8	75
2	—	31	o	o	—	—
5	—	32	o	o	—	—
10	—	31	o	o	—	—
25	—	22	o	o	—	150
50	—	20	VL	o	—	—
100	—	18	L	o	—	—
200	—	17	o	H	—	425
Ni specific						
0	2.5	28	M	M	153	—
2	2.5	28	M	M	—	—
5	2.5	26	M	M	—	—
10	2.5	26	M	M	—	—
25	2.5	23	M—	L	89	100
50	2.5	21	M—	L	—	—

Plants were normal with solutions containing up to 10 p.p.m. aluminium. With 25 p.p.m. plants were small but without distinctive symptoms. With 50 p.p.m. leaves were occasionally very slightly chlorotic (the chlorosis being diffuse), and with 100 p.p.m. chlorosis was more common though still slight. When 200 p.p.m. aluminium were supplied, the plants were stunted and the leaves were narrow and dark blue. Roots were smaller than normal with concentrations of aluminium up to 100 p.p.m. and stunted at the 200 p.p.m. level.

Typical toxic symptoms and chlorosis occurred only at the higher levels of aluminium, though growth was reduced at lower levels. The effect of aluminium was specific and the concentration of aluminium in the leaves of affected plants was higher than normal. The presence of aluminium in the nutrient solution along with nickel reduced the severity of the nickel symptoms in the plants.

The most prominent change produced in the plant nutrient status by aluminium was a reduction in the phosphate content of stem extracts of affected plants.

DISCUSSION

Marked differences were observed in the chlorotic symptoms produced by the various elements. Copper and cobalt both produced a prominent longitudinally-striped chlorosis whilst chromium, zinc, manganese, molybdenum and aluminium gave a diffuse form. Nickel induced a striped chlorosis which developed partly into white necrosis and partly into diffuse chlorosis.

The investigation showed that two types of symptoms were produced by trace-element toxicity, as found by other workers.

Sommer (1945) showed that copper was important in plant metabolism for several reasons, one being that it is a component of several enzyme systems. It is possible

that excessive nickel produces necrosis by replacing copper in one or more of these complexes in a way comparable to the production of iron deficiency in the tissues; such an interaction would explain the effect of additional copper in reducing the degree of necrotic symptoms caused by nickel. Vanselow (1952) has stated that addition of excessive nickel to soil appeared to lower the uptake of copper (but not to deficiency levels) by orange seedlings. The symptoms of copper deficiency in oat plants described by Piper (1942) are different from those of nickel toxicity, but Jacks & Scherbatoff (1934) state that 'in cereals, the tips of the leaves turn white or pale green' when copper is deficient; it may be that in nickel toxicity, inhibition of copper function is localized at centres scattered throughout the leaf, resulting in the production of white areas.

It was found that molybdenum, in concentrations up to 5 p.p.m. in the nutrient solution containing 2.5 p.p.m. nickel, gave no reduction in degree of nickel symptoms (chlorosis or necrosis), and that in concentrations above 5 p.p.m., the degree of chlorosis was intensified. This is in direct contradiction to the results of Millikan (1947*b*, 1948, 1950) who on including 0.1–20 p.p.m. molybdenum in the nutrient solution obtained a reduction in the degree of chlorosis produced in flax and other plants by several trace elements (including nickel) and also reduction in specific symptoms produced by excessive manganese. Failure to substantiate Millikan's results is also reported by Hewitt (1948*b*), who found that molybdenum additions intensified the symptoms produced on sugar beet by excessive concentrations of trace elements; moreover, Warington (1951) failed to obtain evidence of interaction between molybdenum and manganese in oats and recorded increased manganese-induced chlorosis in soybean and flax by increased molybdenum supply.

Millikan (1947*b*, 1950) also reports absence of iron-deficiency chlorosis in flax plants affected with molybdenum toxicity, again conflicting with the results found here for oats and those of Hewitt (1948*b*) for sugar beet.

As with copper, the presence of aluminium in the solution along with nickel reduced the degree of nickel symptoms in the plants. This reduction was correlated with a smaller nickel content of the leaves, although the reduced phosphorus status of the leaf with aluminium applications might also have been involved. The nutrient solutions containing aluminium soon developed a precipitate, indicating that precipitation or adsorption might be responsible for reduction in nickel availability. Evidence that this was not so was obtained by filtering off the precipitate, when the nickel concentration in the solution was found to be unchanged. Aluminium was also determined in the filtrate, and its concentration was found to have been reduced from 25 to 0.4 p.p.m.; it seems probable, however, that some of the aluminium in the precipitate was available to the plants. It would be difficult in many cases to diagnose from the aluminium content of the tissue the extent to which aluminium had reduced uptake of nickel, because differences in the aluminium content of affected and unaffected plants may be very small.

Marked reduction in the phosphorous status of plants receiving aluminium is

commented upon by Hewitt (1947, 1948*c*); both Conner & Sears (1922) and McLean & Gilbert (1928) found that high levels of phosphate in the nutrient solution decreased the damage caused by aluminium, suggesting that aluminium toxicity might be due partly to induced phosphorus deficiency. Wright (1943, 1945) found that the total phosphorus content was invariably higher in the roots—and sometimes also in the leaves—of plants receiving aluminium but states that the water-soluble content in the leaves was always low. A reduction in the phosphorus status of the plant is undoubtedly an important result of aluminium toxicity, and this effect was noted in the aluminium-affected plants described here.

Hewitt (1947) noted that in affected plants the aluminium accumulated in the roots where he found that the main damage occurred (see also Barnette, 1923; Ligon & Pierre, 1932; and Singh & Prasad, 1936). It appears then that identification of an aluminium effect on growth and nickel toxicity may not always be easily made from the aluminium content of the leaf, because the root effect may or may not be paralleled by a significant change in the aluminium content of the leaf.

The production of iron deficiency by trace elements has recently been reviewed and discussed by Hewitt (1951). It is generally considered that a probable explanation of the mechanism is the replacement of iron by the trace element in some essential compound and removal of iron from solution in the tissues by the formation of a ferric-phosphorus-organic complex. Hewitt (1951) draws attention to the order of stability of metal-organic complexes (Mellor & Maley, 1947, 1948; Irving & Williams, 1948; Maley & Mellor, 1950), and of the metal-porphyrin complexes (Gannick & Gilder, 1947; Lemberg & Legge, 1949), and points out the similarity between these and the order of trace-element ability to induce iron deficiency.

From the results given here (aluminium being omitted because of solubility difficulties), the order of activity in producing chlorosis, using a milli-equivalent basis for comparison, was as follows:

$$\text{Ni} > \text{Cu} > \text{Co} > \text{Cr} > \text{Zn} > \text{Mo} > \text{Mn}.$$

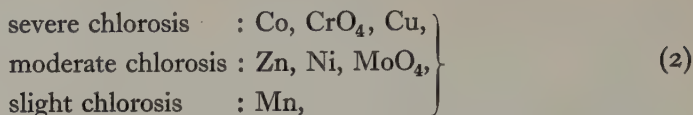
For example:

	Concentration in nutrient solution		Degree of chlorosis
	p.p.m.	m.equiv./l.	
Ni	2.5	0.085	M
Cu	5	0.157	M
Co	10	0.339	M
Cr	10	0.385	L
Zn	25	0.770	VL
Mo	100	2.09	L
Mn	250	9.1	VL

This differs from the order found by Hewitt (1948*b*) for sugar beet in two experiments:

$$\text{Co} > \text{Cu} > \text{Zn} = \text{CrO}_4 > \text{Ni} > \text{Mn}, \quad (1)$$

and



but compares well with the order of stability of metal complexes as given by Hewitt (1951): ^{no sub}



When the over-all toxic effect of a trace element, as estimated by reduction in the height of the plants, was considered, the order of activity of the elements was found to be the same as that for chlorosis:

	Approximate concentration in nutrient solution at which plant height markedly reduced	
	p.p.m.	m.equiv./l.
Ni	2.5	0.085
Cu	12	0.376
Co	15	0.509
Cr	15	0.577
Zn	100	3.08
Mo	200	4.17
Mn	500	18.2

When the activity of the elements in producing specific toxic action was considered, the following order resulted:



This is very different to the order of stability of metal complexes, but is similar to the activity order found by Hewitt (1948*b*) for sugar beet:



Arrangement of the elements on this basis seemed unlikely to be of value in view of the multitude of factors involved in their specific action. For example, in this experiment the phosphorus content of the plants was sometimes lowered by the toxicity, whilst at other times it was increased; also some toxicities were accompanied by induced nitrogen deficiency while others were not. Arrangement on a chlorosis-producing basis is much more promising because in chlorosis only one effect is involved, namely, induced iron deficiency. Hewitt (1948*a, b*), however, has pointed out that all elements may not act similarly in producing chlorosis so that arrangement even on this basis might not have any theoretical significance.

With the possible exception of chromium and aluminium, the diagnosis of heavy metal toxicity by consideration of visual symptoms and the composition of the leaf tissue should not be difficult. In the case of nickel and cobalt, quite apart from the slight visual differences which occur, a high concentration of cobalt in the plant is essential for cobalt toxicity. For diagnostic purposes a relatively low level of

chromium in the leaves would be reflected in reduced vigour, and oat plants (at the same stage of development as those in this experiment) containing more than 1 p.p.m. chromium in the dry matter of the leaves would be suspect even though no specific symptoms are present. Diagnosis of interaction between nickel and chromium would be difficult unless the chromium action were very marked. This method has been applied successfully by one of the authors (Vergnano, 1952) to the determination of trace-element toxicities in serpentine soils. Analysis of the soils themselves was not satisfactory in every case, and diagnosis was most satisfactorily made from pot experiments with oat plants where symptoms were observed and the trace-element contents of the leaves determined. The oat plant is a suitable indicator especially where nickel toxicity is suspected, because it is relatively susceptible and displays characteristic symptoms. The procedure adopted is as follows: a soil:sand mixture is prepared—with a sufficient proportion of sand to give adequate drainage (usually 7 soil:3 sand)—and a low level of nutrients* added to part of the mixture and a higher level† to the remainder; oat seeds are then sown and the plants grown to about the same stage as those described in this paper; symptoms are observed, the trace-element contents of the leaves determined and results compared with those given here. When four Aberdeenshire and two American ultra-basic soils were examined for trace-element toxicities in this way, four were found to contain toxic concentrations of nickel and one a toxic concentration of chromium.

The trace element analyses (except those of aluminium) were supplied by Dr R. L. Mitchell, Department of Spectrochemistry, The Macaulay Institute for Soil Research, to whom the authors are greatly indebted.

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* N 50, P 25, K 50, Ca 250 p.p.m.

† N 200, P 100, K 200, Ca 1000 p.p.m.

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THE VEGETATIVE DEVELOPMENT OF THE POTATO PLANT

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(With 3 Text-figures)

A study of the vegetative growth of potato plants indicated that all varieties grown under identical conditions are likely to show similar growth rates during the early stages of development.

Differences in total leaf area per plant between varieties became apparent at the stage when rapid development of axillary shoots from the main axis occurred. The maincrop varieties, Stormont Dawn and King Edward, attained a greater total leaf area than the early varieties, Ulster Chieftain and Arran Pilot, because of the greater development of axillary shoots.

It is suggested that the number of nodes produced beneath the terminal inflorescence on the main axis approximates to a constant value, independent of variety. Varietal differences were manifested in the distribution between leaves above ground and stolons below, the early varieties producing fewer leaves above ground than the maincrop, in inverse proportion to the number of underground stolons.

The yields of tubers per plant are given for four varieties. Differences between varieties in the rate of tuber formation are related to differences in the development of haulms.

It is considered probable that the potato plant exhibits a basic growth form which is modified in each variety in a characteristic manner according to the stage in the growth cycle and the rate at which the growth substances are distributed to the various organs of the plant.

A recently undertaken study on the relations between plant growth and colonization by potato aphids demanded details of the development of the vegetative phase of experimental potato plants. Observations were made on the increase in leaf area of seven potato varieties during 1950 and 1951, the results of which have been summarized in a previous paper (Taylor, 1952). In 1952 a further study on the vegetative growth of several potato varieties was undertaken, the main purpose being to discover if varietal differences in the vegetative growth rates of axillary shoots could be related to varietal differences in infestations by aphids.

Four of the potato varieties grown in the 1950-51 trials were chosen because of their widely varying growth habits. These were:

- Ulster Chieftain: a low, compact grower, with large, thin, waxy leaflets which tend to roll towards maturity; secondary leaflets are small; a First Early.
- Arran Pilot: of medium height, with strong stems and medium-sized leaflets; secondary leaflets fairly numerous; spreading habit towards maturity; Early.
- Stormont Dawn: fairly tall with strong stems and large, thick leaflets; secondary leaflets not frequent; leaves closely overlap giving good ground cover; Maincrop.
- King Edward: tall and erect; leaflets small, glossy and narrow; numerous secondary leaflets; mature plant with sparse lower foliage but with crowded top growth; Maincrop.

Details of the vegetative growth rates and yields of these varieties, based largely on analysis of the 1952 trial, are given in this paper.

EXPERIMENTAL PROCEDURE

The trial was set out in the form of a 4×4 Latin square, all tubers being planted on the same day. Each plot contained 150 plants—fifteen drills with ten plants per drill. The seed tubers were Scotch seed (certified 'A'). Soon after emergence above ground each plant was pruned to a single stalk, and this operation was carried out, as necessary, throughout growth. Few tuber lateral-shoots were produced on Ulster Chieftain even in the early stages of growth and none after the beginning of tuber formation. King Edward continued to send up lateral-shoots until well after the appearance of the flower buds; Stormont Dawn and Arran Pilot were intermediate in shoot production. As a result of pruning, each plant developed a single main stem with axillary shoots produced from the nodes. Samples of leaves were taken from the basal, lower, middle, upper and apical zones of the main stem, as defined below.

In a well-developed potato plant the greater portion of the five or six first-formed leaves on the main axis form a cover over the ground. The lowest leaves (generally two) which actually touch the ground are defined as 'basal'. The 'lower' leaves are the next three and are generally large, forming a canopy over the ground without touching it except possibly at their tips. Next are the 'middle' leaves, which are fully expanded or nearly so and whose laminae are more or less at right angles to the main axis. Near the top of the shoot of a young plant are one or two rudimentary leaves which are just beginning to expand from the tightly packed terminal rosette of leaves and flower buds. These are the 'apical' leaves and between them and the 'middle' leaves are the expanding 'upper' leaves.

In all the varieties studied the first axillary shoots to develop were in the nodes towards the base of the main stem, but later, soon after the appearance of the terminal rosette, axillary shoots developed from the uppermost nodes. As many as seven or eight strongly growing basal axillary shoots may branch from the main stem above ground in a variety such as King Edward with usually two (but sometimes three) upper axillary shoots. Measurements of upper, middle and lower leaves of the shoots were recorded separately for each shoot according to the position of the node on the main stem from which it originated.

The experimental area was planted on 24 April, with 24 in. between the ridges and 18 in. between the tubers. Ridging-up was carried out on 6 June, but each plant in the trial was inspected within a few hours after the operation and soil removed from the lower leaves where necessary.

The date of emergence above ground of each variety was taken as the day on which 50% of the plants which had appeared above ground were expanding their first leaves. Leaf area estimates were made at intervals of 10 days from the date of emergence of each variety, except during the early stages of development when

sampling was more frequent. At each sampling, measurements were taken of ten leaves from each zone on the main stem and twelve leaves from every third axillary shoot for each variety, the plants and leaves being selected at random. In addition, two plants were selected from each plot and the number and type of leaf on the main stem and on each axillary shoot was recorded, together with the terminal inflorescence if present.

INCREASE IN TOTAL LEAF AREA

Table 1 gives the total area in sq.cm. converted to \log_{10} for the four varieties used in the 1952 trial. These values plotted against time (measured as the number of days after emergence) show that during the early stages of development when

TABLE 1. *Mean leaf areas (sq.cm. — \log_{10} values) per plant*

(Areas of main shoots (M), axillary shoots (A) and the sum of the two (T).)

Variety	...	Ulster Chieftain	Arran Pilot	Stormont Dawn	King Edward
Mean date of emergence	...	20. v. 52	19. v. 52	22. v. 52	22. v. 52
Date of sample					
22. v	M	1.42	1.73	1.54	1.54
	A	—	—	—	—
	T	1.42	1.73	1.54	1.54
25. v	M	1.54	1.92	1.70	1.68
	A	—	—	—	—
	T	1.54	1.92	1.70	1.68
1. vi	M	1.99	2.41	2.20	2.22
	A	—	1.18	0.85	1.04
	T	1.99	2.43	2.22	2.24
7. vi	M	2.49	2.77	2.62	2.61
	A	0.60	1.79	1.72	2.10
	T	2.50	2.82	2.67	2.72
15. vi	M	2.94	3.15	3.15	3.12
	A	1.30	2.37	2.31	2.45
	T	2.95	3.21	3.24	3.26
25. vi	M	3.24	3.32	3.36	3.21
	A	1.67	2.71	2.90	3.13
	T	3.25	3.42	3.49	3.49
5. vii	M	3.32	3.38	3.47	3.37
	A	2.10	3.05	3.21	3.32
	T	3.35	3.55	3.66	3.65
15. vii	M	3.30	3.26	3.24	3.32
	A	2.10	3.27	3.37	3.47
	T	3.33	3.57	3.69	3.70
25. vii	M	3.17	3.06	3.24	3.14
	A	2.05	3.27	3.37	3.37
	T	3.20	3.48	3.61	3.57
14. viii	M	2.74	2.65	2.94	2.76
	A	1.83	2.93	3.31	3.14
	T	2.79	3.11	3.46	3.30

growth is largely vegetative, the rates of increase in leaf area are very nearly equal for all varieties (Fig. 1). Ulster Chieftain, from the first estimate of leaf area, had smaller haulms, but, by shifting the points for this variety on the time scale, it can be seen that they follow approximately the same growth curve as the other varieties.

A plant was recorded as having emerged when two of the leaves had begun to expand, giving a total area of approximately 70 sq.cm. Since the emergence for

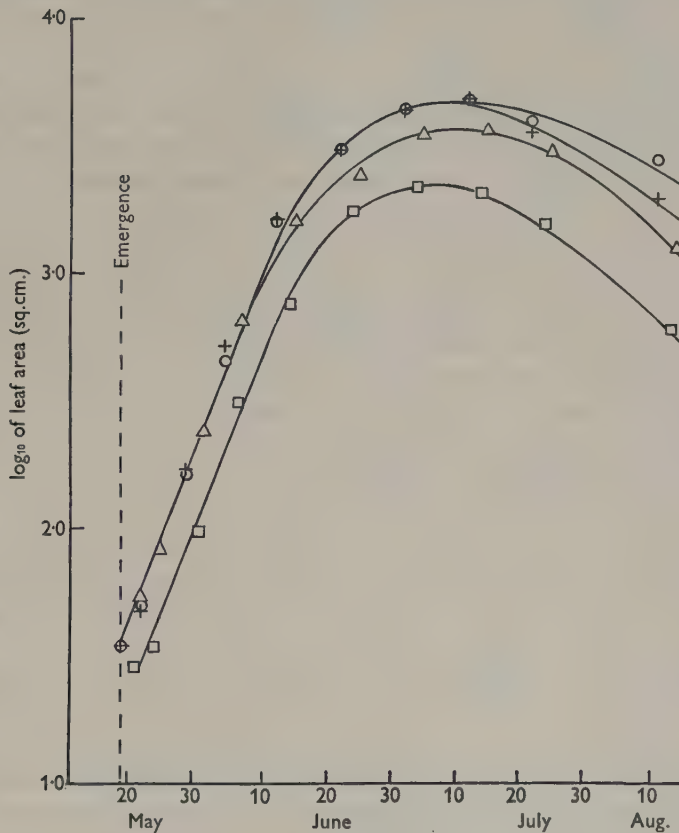


Fig. 1. \log_{10} of total leaf area (sq.cm.). \square = Ulster Chieftain; \triangle = Arran Pilot; \circ = Stormont Dawn; $+$ = King Edward. Positions on the time scale are corrected for emergence by shifting the values for a variety back according to the mean number of days after 19 May (the emergence of Arran Pilot) that the variety emerged (see Table 1).

each variety was recorded as the day on which 50% of the plants had reached this stage, the leaf area at emergence was 35 sq.cm. or \log_{10} 1.54. The values for Ulster Chieftain were somewhat lower.

After the inception of flowering, the maincrop varieties, King Edward and Stormont Dawn, grew more rapidly than the early varieties, Ulster Chieftain and

Arran Pilot. The first flower buds were noted on Arran Pilot on 12 June, 24 days after the shoots emerged from the ground, and the three other varieties about 4 days later. By the 42nd day after emergence the flowers terminating the main stems of Stormont Dawn were fully open, the flower cymes of Arran Pilot were mature and in many cases had produced flowers, and the 'blind' buds of King Edward were well developed. The flower buds of Ulster Chieftain usually remained very small and undeveloped and were largely hidden by one or two terminal leaves which never expanded. A few 'bolters' of this variety produced flower cymes, but these were ignored in the sampling.

With the initiation of the terminal flower cyme the number of leaves on the main axis is fixed, and thereafter development in leaf area consists of expansion of existing leaves and extension of the axillary shoots. The development of the main shoot and the axillary shoots will, for convenience, be discussed separately.

DEVELOPMENT OF THE MAIN SHOOT

The development of the main shoot was similar for the four varieties studied, the leaves produced successively on the stem following the typical S-shaped curve of growth. The first two leaves produced at the base of the stem were generally small and soon died, except in Ulster Chieftain where some of these primary leaves were of appreciable size and obviously played a part in assimilation. Leaves were produced rapidly until the appearance of the terminal inflorescence about 3 weeks after the emergence of the plant. About half the leaves at this time were large and these, with the remainder on the main stem, continued to expand for some time, although the rate of expansion decreased considerably as the plant neared maturity and the axillary shoots began their expansion. Consequently the leaves produced just before the terminal inflorescence often remained small and, in some cases, rudimentary.

The number of nodes produced on the main stem above ground is said to vary very little from one variety to another (Bald, 1944). Werner (1934) gives the figures 15-19 leaves with axillary buds above ground, and 10-14 nodes from which stolons may develop below ground as the normal complement for Triumph variety, while Bald & Hutton (1950) obtained a mean of 15.2 for the number of nodes above ground independent of the varieties studied.

The mean numbers of nodes for the four varieties studied at Sutton Bonington are given in Table 2. The number of nodes above ground within each variety varied to some extent but, for the conditions of the experiment, there were significant differences between all varieties except between Arran Pilot and Ulster Chieftain (cf. Bald, 1944). The figures for 1951 show a significant difference between varieties with the exception of Stormont Dawn and Arran Pilot.

The numbers of stolons per plant, given in Table 3, are obtained from counts on four plants of each variety made on 26 June and 15 July, between which dates flowers (or flower buds) were well formed. This gives a value for the number of

nodes on each main axis between 26.0 and 26.7. Since the phyllotaxy of the potato plant is 13/5, this represents two complete cycles of nodes. Thus it appears that, although the number of nodes on the main axis approximates to a constant value, independent of variety, the distribution between leaves above ground and stolons below varies according to the inherent characters of each variety, the early varieties tending to produce fewer leaves above ground, in inverse proportion to the number of underground stolons, than the maincrop.

AXILLARY GROWTH

Rudimentary axillary leaves were noted in the nodes of the main axis within a week of the emergence of the main stem from the soil, but increase in area did not appear to take place until about 12 days after emergence in King Edward; in Arran Pilot and Stormont Dawn expansion of axillary leaves was delayed until about 16 days after emergence. By the time flower buds had been formed on the main axis (approximately 24 days after emergence) the axillary shoots had become quanti-

TABLE 2. *Mean number of nodes above ground on the main stems of four potato varieties*

Variety ...	Ulster Chieftain	Arran Pilot	Stormont Dawn	King Edward
1951				
No. of nodes	13.8	16.1	16.4	18.3
S.D.	1.81	1.36	1.05	1.16
1952				
No. of nodes	14.2	14.5	15.3	17.7
S.D.	0.65	1.35	1.04	0.77

TABLE 3. *Mean number of stolons on mature plants of four potato varieties*

Variety ...	Ulster Chieftain	Arran Pilot	Stormont Dawn	King Edward
26. vi	11.3	12.0	11.0	8.5
15. vii	12.3	11.4	10.6	9.5
Mean	11.8	11.7	10.8	9.0

tatively important in relation to the total leaf area. In Ulster Chieftain axillary leaves were mostly rudimentary, although one or two small shoots did develop from the lower nodes in most cases.

In all varieties the first axillary shoots to develop were in the basal nodes of the main stem, the number of well-developed shoots differing with each variety. In the mature King Edward plant the basal axillary shoots averaged 7.5, 6.5 in Stormont Dawn and 5.6 in Arran Pilot. On each fully developed axillary shoot the average number of leaves below the terminal inflorescence was 10, the average number of nodes being 13. At some time before the lower group of axillaries were fully developed axillary shoots extended from the nodes just below the terminal inflorescence of the main axis. Usually two shoots were well developed with one

becoming dominant. From the remaining nodes of the main stem there was very little development of axillary shoots in any variety.

Terminal inflorescences developed on the lower axillary shoots about 23 days after the beginning of their rapid development. With their inception two or three 'secondary' axillary shoots were produced in the nodes just below the inflorescence, but none was produced from the lower nodes. These 'secondary' axillary shoots remained comparatively small.

Ulster Chieftain differed from the other varieties in showing little development of axillary shoots. At the most, two or three shoots were developed from the lower

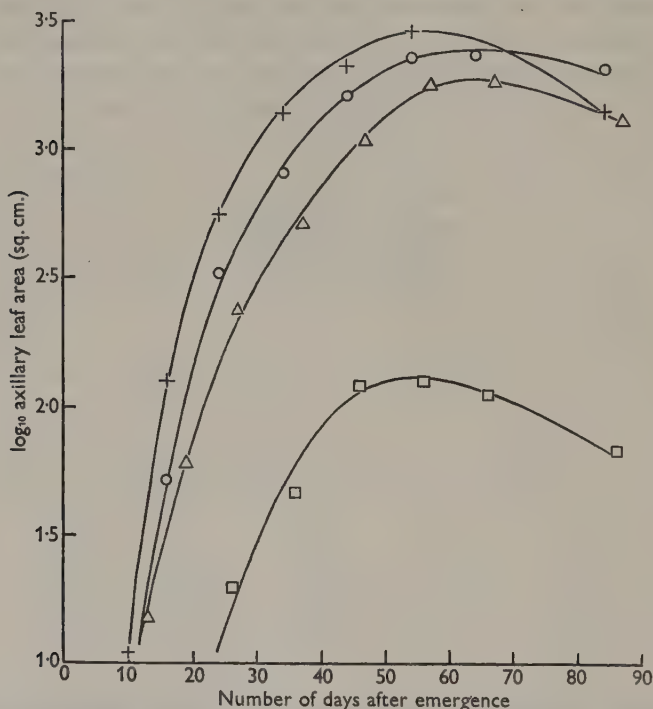


Fig. 2. Growth of axillary leaves. □ = Ulster Chieftain; Δ = Arran Pilot; O = Stormont Dawn; + = King Edward. The time scale represents the number of days from the mean emergence date of each variety.

nodes of the main stem and production of axillary shoots from the upper nodes was rare. The number of leaves per axillary shoot averaged 3.3 and at the stage of their maximum development they constituted only 6% of the total area of the foliage. When the leaves of the main stem had fully expanded, the axillary leaves constituted 36% of the total leaf area in Stormont Dawn, 47% for King Edward and 32% for Arran Pilot. Later the percentage increased as the leaves of the main stem died, while those on the axillary shoots continued to develop.

In Fig. 2, growth curves of the axillary shoots show a divergence in growth rates for each variety almost from the first. The axillary shoots on the maincrop varieties attain their greater area by growing faster than those on the early varieties rather than by beginning their growth earlier. This increased growth rate on the part of

TABLE 4. *Estimated maximum weight of tubers per plant and time of 50% and maximum yields*

Variety	...	Ulster Chieftain	Arran Pilot	Stormont Dawn	King Edward
Maximum weight (g.)		339	912	1043	1043
Time of 50 % yield*		40	59	73	73
Time of maximum yield*		64	74	85	85

* Measured as the number of days from the mean-emergence date of each variety.

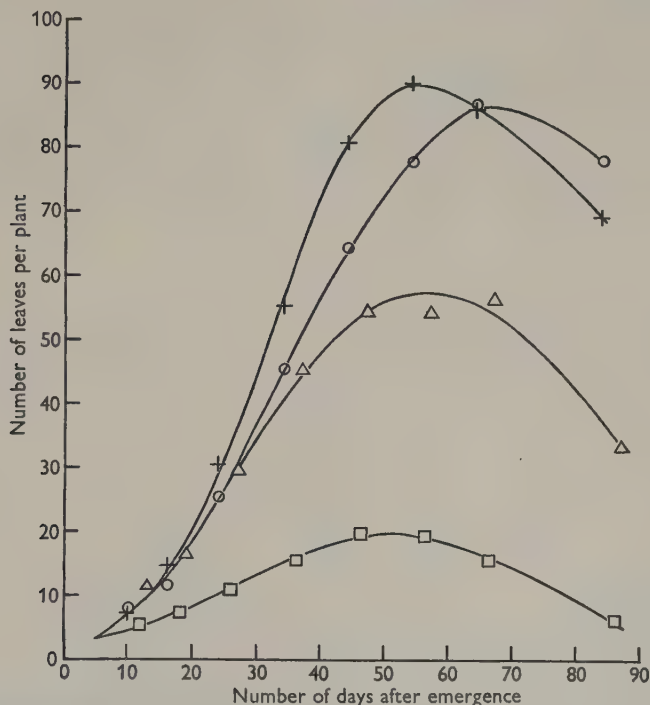


Fig. 3. Number of leaves per plant. □ = Ulster Chieftain; △ = Arran Pilot; ○ = Stormont Dawn; + = King Edward. The time scale represents the number of days from the mean emergence date of each variety.

the maincrop varieties (Stormont Dawn and King Edward) is associated with a greater number of axillary shoots produced and thus a greater number of leaves per plant (Fig. 3).

The leaf areas of axillary shoots developed from the upper nodes of the main stem showed no appreciable differences in growth rates between varieties. The time of

appearance of these shoots, however, differed to some extent between varieties. Those on King Edward appeared last among the varieties examined; this delay is probably associated with the greater number of axillary shoots produced from the lower nodes.

TUBER FORMATION

The yield of any potato plant is limited by numerous environmental factors such as water supply, soil type, amount and type of fertilizer, spacing of setts, etc. Under similar environmental conditions the yield of any variety can be considered to be dependent largely on the growth form of the plant if one assumes the metabolic efficiency of different varieties to be similar. This assumption can be illustrated, but not proved, by reference to yield data obtained from the four varieties studied in this experiment.

TABLE 5. *Number and weight of tubers per plant*

Variety	Ulster Chieftain					Arran Pilot				
	Ti.	Ch.	Sw.	Total no.	Total weight (g.)	Ti.	Ch.	Sw.	Total no.	Total weight (g.)
Date of sample										
12. vi	4	4	2	10	14	5	3	1	9	6
26. vi	5	3	4	12	163	5	2	6	13	163
15. vii	4	1	6	11	232	2	2	7	11	422
14. viii	2	2	5	9	333	1	2	8	11	838
26. viii	1	3	6	10	341	0	2	9	11	896

Variety	Stormont Dawn					King Edward				
	Ti.	Ch.	Sw.	Total no.	Total weight (g.)	Ti.	Ch.	Sw.	Total no.	Total weight (g.)
Date of sample										
12. vi	6	1	0	7	1	5	0	0	5	—
26. vi	11	4	3	18	50	6	6	1	13	41
15. vii	4	8	6	18	235	6	6	4	16	230
14. viii	4	6	8	18	655	4	7	8	19	643
26. viii	2	7	10	19	861	3	6	9	18	849

Ti.=Tuber initials. Ch.=‘Chats’—less than $1\frac{1}{4}$ in. diameter. Sw.=Seed size and above, i.e. greater than $1\frac{1}{4}$ in. diameter.

Four normal, healthy plants of each variety were carefully lifted on five successive dates during the period of the trial. The tubers were cleaned, weighed and counted (Table 5). The numbers of stolons and tuber initials on each plant were also noted.

In the two maincrop varieties, King Edward and Stormont Dawn, the formation of tubers was delayed until the rapid extension of axillary shoots had almost been completed. In the variety Ulster Chieftain tubers were formed comparatively early in the growth of the plant and little axillary growth took place above ground. Arran Pilot lay somewhere between these two types, production of tubers and formation of axillary shoots being more or less balanced, so that tubers were formed and began to bulk fairly early in the season (although not so early as in Ulster Chieftain).

In terms of partition of growth metabolites (Bald, 1946) this means, in Ulster Chieftain, a high initial tuber partition coefficient, resulting in a comparatively high yield of tubers early in the season, but with the formation of a small haulm. King Edward has a high initial partition coefficient of growth metabolites passing to the foliage and this is manifested in the copious formation of axillary shoots resulting in a large haulm.

The maximum yields for each variety given in Table 4 are estimated by the extrapolation of the curves of increase in yield constructed from the data obtained at each sampling. Ulster Chieftain reached its maximum yield only 10 days before Arran Pilot and 21 days before the maincrop varieties. 50% of the total yield, however, was produced within 40 days after the emergence of the plants from the soil—considerably earlier than comparable percentage yields for the other varieties. This 'early bulking' for which Ulster Chieftain is noted is associated with the high partition of growth metabolites in favour of tuber formation. The relatively long period required to reach the maximum yield from the 50% level is associated with the small haulm produced by the plant, which gives it a small assimilative area. The larger haulms produced by the maincrop varieties, Stormont Dawn and King Edward, although delaying the development of tubers, provide a large area for assimilation and consequently result in relatively high yields of tubers.

DISCUSSION

The basic growth form of the potato plant is probably defined by the production of a main axis terminating in a flower cyme, below which a total of twenty-six nodes have been differentiated. The proportion of above-ground axillary shoots to the number of stolons below ground varies according to the pattern of development of each variety. Thus, in the 1952 trial, an average of 14.2 nodes were formed above and 11.8 stolons below ground in Ulster Chieftain, compared with 17.7 nodes above ground and 9.0 stolons below for King Edward. However, this ratio is not constant for each variety but varies according to the conditions of the environment, as shown by a comparison of the figures for 1951 and 1952 (Table 3). Nevertheless, the proportion of stolons to axillary leaf nodes does give a clear indication of the partition of the growth metabolites, and hence the expected form of growth, at an early stage in the development of the plant. Differences between varieties are later superimposed on this basic pattern by the rapid production of axillary shoots above ground or of tubers below.

Environmental factors such as water supply, temperature and soil conditions, have been shown to affect the growth rates and yields of the potato plant (Glover, 1947; Werner, 1942; Van der Plank, 1946), but, in Great Britain at any rate, the variations in these factors from season to season do not appear to alter the varietal growth form to a great extent. Of the variety trials undertaken in 1950, 1951 and 1952 at Sutton Bonington, although the growth rates for each variety differed from year to year, each variety was affected to more or less the same degree. Thus the

morphological differences between varieties (described in this paper) were always readily apparent, and each variety conformed to its basic pattern of vegetative development.

It is a pleasure to thank Mrs N. McDermott of the University of Nottingham School of Agriculture for her advice and criticism.

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REVIEWS

Limnology, 2nd ed. By PAUL S. WELCH, Ph.D. Pp. 538. London: McGraw Hill. 68s.

Fundamentals of Limnology, 2nd ed. By FRANZ RUTTNER. Translated by D. G. FREY and F. E. J. FRY. Pp. 242. Toronto University Press (London: Geoffrey Cumberledge). 52s.

These two books make an interesting contrast, one failing where the other succeeds. Welch's text-book, although containing additional information, retains the same general mode of presentation as the first edition. The attempt to cover the whole field of Limnology has resulted in an uninspired account with many omissions, and a lack of balance in the amount of space devoted to the various aspects of the subject. For example, very little space is given to rivers and streams, or to freshwater fish, aspects of Limnology which have received a great deal of attention recently.

The detailed classification of habitats and communities is of little value, and nowhere does a clear picture of the plant and animal communities, and their relationships with the environment, emerge. Neither is there any indication of the relative significance in fresh waters of the various animal and plant groups. There are also definite errors of detail: for example, to refer to 'Bacteria and other fungi' is unconventional to say the least, and to include the Endoprocta amongst the Bryozoa is quite wrong.

One thing which makes this a very dull book is the detailed subsectioning of the chapters. This may be justified in a large and comprehensive text-book, but in a book of this type, which is clearly not comprehensive, the effect of the subdivision is one of repeated discontinuity. It is as if the book has been strung together in bits and pieces. This is a pity because Prof. Welch is a limnologist of international repute. Perhaps we may look forward to a third and completely reorganized edition.

Despite these criticisms it is only fair to say that the book is well produced, the diagrams and figures are clear, and the bibliography is extensive.

Ruttner's book succeeds because it confines itself definitely to fundamentals, and although it is a much shorter book and attempts much less, it leaves the reader with a far clearer picture of Limnology than Welch's more ambitious book. The translation has been well done, apart from an occasional unwieldy sentence. The phrase 'enjoyment of light' in figure 39 is curious, particularly when referring to Oscillatoria, and probably originates from faulty translation. An excellent feature is the list of German-English equivalents.

The first part of the book is an account of the physico-chemical conditions, presented in a very readable manner. The remainder of the book deals with the plant and animal communities, and from the point of view of a balanced presentation, too much space is given to plankton and the 'aufwuchs' community. This results in rather a limited account of lakes, apart from the special conditions of ooze communities, and a very slight account of the communities of running water. This over-emphasis of plankton and 'aufwuchs' can be excused because they are subjects on which Ruttner can write with the authority of one who has made a special study of these communities. In any case Ruttner himself refers to this lack of balance by pointing out with justice that of all communities plankton is the most truly aquatic, being entirely dependent on the water which buoys it up. The bibliography is full and up to date, although there are certain omissions, for example, if Storch's work on Crustacean feeding mechanisms is mentioned, Cannon's work ought to be mentioned as well.

Despite these criticisms, however, this is a very useful book, nicely produced with thick, serviceable pages, and clear if somewhat roughish diagrams and graphs.

A point which occurs to me on reading these two books is the alarming extent to which Limnology has congested itself with ponderous scientific terms. Welch's book is the worst offender, but Ruttner is heavily laden with imposing words. Any science will accumulate a terminology but the terms should represent an economy of thought and words—such terms for example as 'thermocline' or 'eutrophic'. One cannot feel, however, that such terms as 'oligoaerobic', 'bioeston', 'hydric limitation' and a host of others serve any useful purpose. Indeed they are dangerous; so often the fine-sounding term gives a false sense of security regarding fundamental ignorance.

Finally, there is another fault common to both books—namely, the omission of the authorities for the species referred to in text and tables. This may seem a quibble, but it is an essential if a specific name is to mean anything.

H. P. MOON

Zoogeography of the Sea. By SVEN EKMAN. Pp. xii + 417, with 121 text-figures and 49 tables. London: Sidgwick and Jackson Ltd. 1953. 42s.

A request by the editor of 'Text-books of Animal Biology' to Prof. Sven Ekman to make available in an English edition his *Tiergeographie des Meeres* afforded an opportunity of revising and bringing up-to-date this admirable survey of the geographical distribution of the fauna of the seas. It is much more than a mere catalogue of organisms occurring in each geographical region: it attempts a causal analysis of present-day knowledge of the distribution of organisms. Of the 374 pages of text 310 are devoted to a study of the benthic fauna. The chapter headings indicate the range of this survey: the warm-water fauna of the tropical continental shelf; the boreal fauna of the north Atlantic; the temperate fauna of the north Pacific; the arctic fauna; the warm-temperate faunas of the southern hemisphere; the antiboreal faunal regions and the antarctic; the benthic deep sea fauna and a chapter on longitudinal distribution and bipolarity.

A reference to two chapters will illustrate the scope of the treatment of each area. Thus an account of the warm-water fauna of the tropical continental shelf emphasizes the richness of the Indo-Malayan region as compared with the poverty of Atlantic regions. However, Ekman refuses to accept the view that the present paucity of the Atlantic fauna is due to its emergence from the Indo-Malayan region as an 'impoverished offspring'. The richness of the Indo-Malayan region is due to the fact that it represents a descent from a rich homogeneous Tethys fauna whilst the Atlantic suffered from a deterioration of climate with its consequent effect on the fauna. The zoogeographer must thus consider palaeozoology, palaeogeography and palaeoclimatology before he can reach conclusions regarding the present-day distribution of the fauna, as the fauna of to-day is the result of long interaction between animate and inanimate nature. Zoogeography must proceed historically and regard 'time' as a sort of fourth dimension in the whole of nature.

A discussion of the boreal fauna of the North Atlantic, probably the best known of all marine faunas, enables the author to correlate its distribution with such factors of the environment as temperature and salinity. Any attempt at an explanation of faunal distribution must include a causal analysis of the interaction between the physiology of the animal and its external environment. However, this interaction may not remain constant during the whole lifetime of the animal, and it is possible to 'distinguish between a *reproductive* eurythermy or stenothermy which occurs during the reproductive period and a *vegetative* eurythermy or stenothermy at other times of life'.

The science of zoogeography must therefore combine many disciplines. Fundamentally an adequate taxonomy is essential. Ecology must be linked with its fundamental science of physiology. It requires a methodology with a knowledge of oceanography, present and past climatology, geomorphology and palaeontology.

The pelagic fauna does not receive such an intensive survey as the benthic but the main features of the distribution of its fauna are adequately covered. The pelagic regions are shown to be less isolated than those of the shelf regions and often characterized by species whose genera occur in other main regions. The pelagic regions are 'more weakly characterized taxonomically'.

Throughout the book Ekman has emphasized that a zoogeographical region is characterized more by an endemic family than by an endemic genus and that an endemic genus is more important than an endemic species and that endemic elements are more important than those which occur in neighbouring regions. Also that a parallelism can be drawn between a taxonomic system of classification and a zoogeographical system of regions, both of which attempt an historical account of the fauna of the world with continuous change in time.

The book is well illustrated with numerous tables and maps and drawings of representative species. A bibliography of 598 references indicates the extensive scope of the survey.

E. E. WATKIN

Physiology of Seeds. By W. CROCKER and L. V. BARTON. Pp. 267. Waltham, Mass.: The Chronica Botanica Co.; London, W.C. 2: Wm. Dawson and Sons, Ltd. 1953. \$6.50.

This book contains a wealth of information on all branches of seed research. Chapters are devoted to the biological aspects, including anatomy, seed production, causes of dormancy and methods of breaking it, the chemical composition of seeds, the food reserves, the changes occurring during germination and the enzymes concerned. The external conditions affecting seeds during storage and germination are also examined, and there are chapters on vernalization, embryo culture and seed-borne diseases. For each topic discussed, all the available information is ably summarized and ample references are given to enable the reader to follow up any line that interests him. Emphasis is placed on text-books or papers containing useful explanations of technical terms, expositions of fundamental principles or accounts of methods. Cross-references to other chapters bearing on the subject are provided.

Having dealt as fully as possible with each subject the authors indicate where further research is required. The practical applications are also discussed and these cover a wide field, from the food value of seeds to horticultural methods of handling dormant seeds and the use of linseed oil in the manufacture of paint. In fact, an amazing amount of useful and interesting information is presented, each fact taking its appropriate place in the whole story of the physiology of seeds. A detailed table of contents gives page references to the subsections under each chapter heading and there is a comprehensive index. These make it easy to locate the paragraphs dealing with such diverse subjects as, for example, the improbability of germinating 'mummy' wheat from an Egyptian tomb or the likelihood of seed-corn being affected by an atomic explosion.

This book should prove invaluable to all research workers using seeds and to students of plant physiology and biochemistry; many others whose work or hobby is concerned with seeds will find the lucid text interesting and helpful. As the volume seems likely to be used so much, it is a matter for regret that many paragraphs, apparently selected at random, have been printed in rather irritating small type.

JOAN THURSTON

Insect Physiology. Edited by K. D. ROEDER. Pp. xiv + 1100. New York: John Wiley and Sons, Inc.; London: Chapman and Hall Ltd. 1953. 120s. net.

The mounting interest in insect physiology over recent years as opposed to, say, echinoderm physiology, must in part be ascribed to the economic importance of this group. Yet, as much fundamental knowledge continues to be accumulated without a thought as to its

possible application, it is also clear that insects have come to be regarded as eminently suitable material for many experimental investigations. A decade or so ago the task of surveying this rapidly expanding field might not have appeared too formidable. Now that the stream of original papers has become a flood, many insect physiologists (whose studies also tend to become increasingly specialized) would feel some diffidence in undertaking an authoritative commentary on the whole discipline. This difficulty has been overcome in the present volume—the third comprehensive treatise on insect physiology—by joint authorship. Of the fifteen contributors, twelve are American, three Australian. Each is a specialist in his own field.

To those already familiar with their researches, the following brief résumé of the contributors and their subjects may help to convey something of the authority and flavour of the book. The arrangement is by organ system: cuticle (three chapters by A. G. Richards); respiration (two chapters by G. A. Edwards); the blood (two chapters by J. B. Buck); haemocytes and pericardial cells (one chapter by S. C. Munson); digestion (four chapters by M. F. Day and D. F. Waterhouse); nutrition (one chapter by W. Trager); excretion (one chapter by R. L. Patton); nervous system (two chapters by K. D. Roeder); vision, mechanical and chemical senses (three chapters by V. G. Dethier); insect flight (three chapters by L. E. Chadwick); behaviour (four chapters by T. C. Schneirla); growth and development (four chapters by D. Bodenstein). There is also an unexpected, but none the less welcome, chapter on the biochemistry of muscle by D. Gilmour.

The treatment of the subject-matter by most of the authors has been notably selective, major pieces of research being singled out as models for detailed discussion. This method proves excellent in presenting principles clearly to the reader and has the added advantage that the text does not become clogged by references. Nevertheless, this has also meant that the bibliography, although lengthy, is by no means exhaustive. Perhaps, inevitably, the writing shows some unevenness. Many of the chapters are very good indeed. For example, the account of insect flight is surely the best yet written, and several other sections also reach this high standard. Although it seems to the reviewer that a few chapters are needlessly obscure, this is a small price to pay for the richness and diversity of outlook which is presented to us. It remains to say that the book is handsomely printed and that the 257 figures, mainly line drawings, are excellent.

The advanced student and research worker in this field will undoubtedly regard this volume as an indispensable work of reference, although the price will unfortunately place it beyond the pockets of many in this country. Entomologists engaged in insecticide research will find that their interests have been remembered since space has been devoted to such topics as the mode of action of drugs and enzyme inhibitors. At the same time, applied entomologists reading these pages may well alight on a new and stimulating approach to their problems. This is surely one of the major functions of the science of insect physiology.

A. D. LEES

Legumes in Agriculture. By R. O. WHYTE and H. C. TRUMBLE. Pp. 367. F.A.O. Agricultural Studies no. 21. Rome: Food and Agriculture Organization of the United Nations. 1953. 15s.

The recovery and maintenance of soil fertility is the most urgent problem facing world agriculture at the present time. Monoculture and shifting cultivation have made large areas of the world surface derelict and subject to wind and water erosion. Outside the humid temperate zone there are vast areas where stable systems of crop culture have not been developed and where there is a pressing need for education, research and positive direction in the principles of sound crop husbandry and soil conservation. In this connexion, the use of leguminous plants can assist above all else in restoring and maintaining the fertility of outworn soils. The search for suitable species and their utilization is only just beginning and,

owing to restricted facilities for research in the under-developed areas, the effort now being made is not commensurate with the magnitude of the problem. 'The tropics are, in general, 100-150 years behind the humid temperate zones in understanding the significance of legumes in maintaining soil fertility and in feeding animals.' The problem is not merely that of application of established techniques from the temperate areas; it is far more basic than this, and the conditions encountered are far more complex than any existing in the highly developed areas of temperate latitudes.

This volume presents for the first time a factual survey of the agronomic status of leguminous species and summarizes their present and potential use in crop husbandry over most of the World. Experiences of tropical workers are presented on the basis of country or state, and although the data available are often meagre and scrappy, the information will serve as an extremely useful guide to the testing and introduction of species. It is regrettable that, owing to gaps in our knowledge, very little could be said in this work on problems of seed production in tropical legumes. Successful plant introduction and particularly plant adaptation, even in the tropics where vegetative propagation of crops is being utilized on a large scale, can be accomplished very much more quickly and economically if adequate seed supplies are available.

Part II of the work gives a valuable account of the botanic and agronomic characters of the important genera, species and strains within the family.

The volume will undoubtedly be of greatest value as a concise handbook of leguminous species in agriculture to those isolated groups of agricultural research workers in the tropical and subtropical zones who are struggling with the unspectacular and intricate problems of plant introduction, tests of seed types from different countries and rotations. It should also be consulted by the student of agriculture in the temperate areas if only to convince him of the favourable conditions in which he works.

WATKIN WILLIAMS

Plant Diseases. By F. T. BROOKS. Second edition. Pp. xiii + 457. London: Oxford University Press. 1953. 38s. net.

The re-written *Plant Diseases* has 100 more pages and 70 more words per page than the first edition (reviewed in *Ann. appl. Biol.* 16, 486). Virus diseases, now grouped under hosts, are given 37 pages instead of 16, bacteria (16 pages) are classified under genera, and the account of deficiency diseases has been expanded.

In the main, however, the new edition, like the old, deals with the fungi parasitic on plants of economic importance and with the symptoms that they induce. Major additions have been made in the chapter on the Hyphomycetes, which has grown from 31 pages to 49. Changes due to the increased prominence since 1928 of specific diseases are exemplified by the treatment of antirrhinum rust, banana-leaf spot, blister blight of tea, Dutch elm disease, sooty blotch of sycamore and South American leaf disease of rubber.

Plant Diseases is not, and does not claim to be, a text-book of plant pathology, but it has proved itself the one book that the practising plant pathologist cannot do without. Its style is the man-matter-of-fact, to the point, packing a maximum of information into a minimum of words.

Dr Dowson, Dr Garrett and Dr Robertson, who, after Prof. Brooks's death, shepherded the new edition through its final stages, have completed a memorial worthy of its author.

R. W. MARSH

